Introduction to mAdb

Esther Asaki (*in absentia*), John Powell, & John Greene, Ph.D.

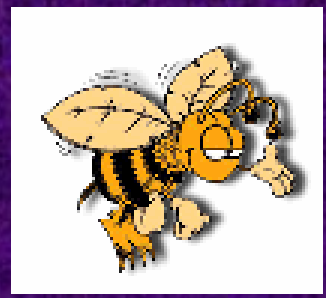
I. Introduction & Overview of the mAdb system

II. Managing Projects & Uploading Arrays

III. Initial array quality analysis

IV. Creating datasets and additional filtering

V. Basic data analyses and dataset management



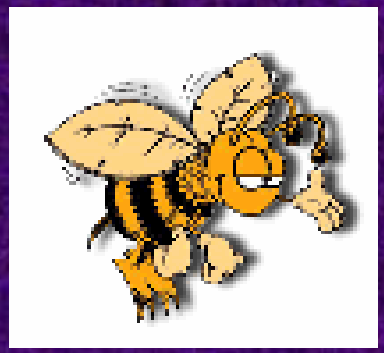
September 28, 2004

Logging into the Training Server

- Point your browser at http://madb-training.cit.nih.gov – for use in class only!
- Your username is on the card on your desk
- Today's Password is on whiteboard near door
- Don't request a mAdb account on the training server!! – request at madb.nci.nih.gov or madb.niaid.nih.gov
- Do not maximize your browser; leave room to see and click on other windows

I. Introduction & Overview of the mAdb system

mAdb BioInformatics Project



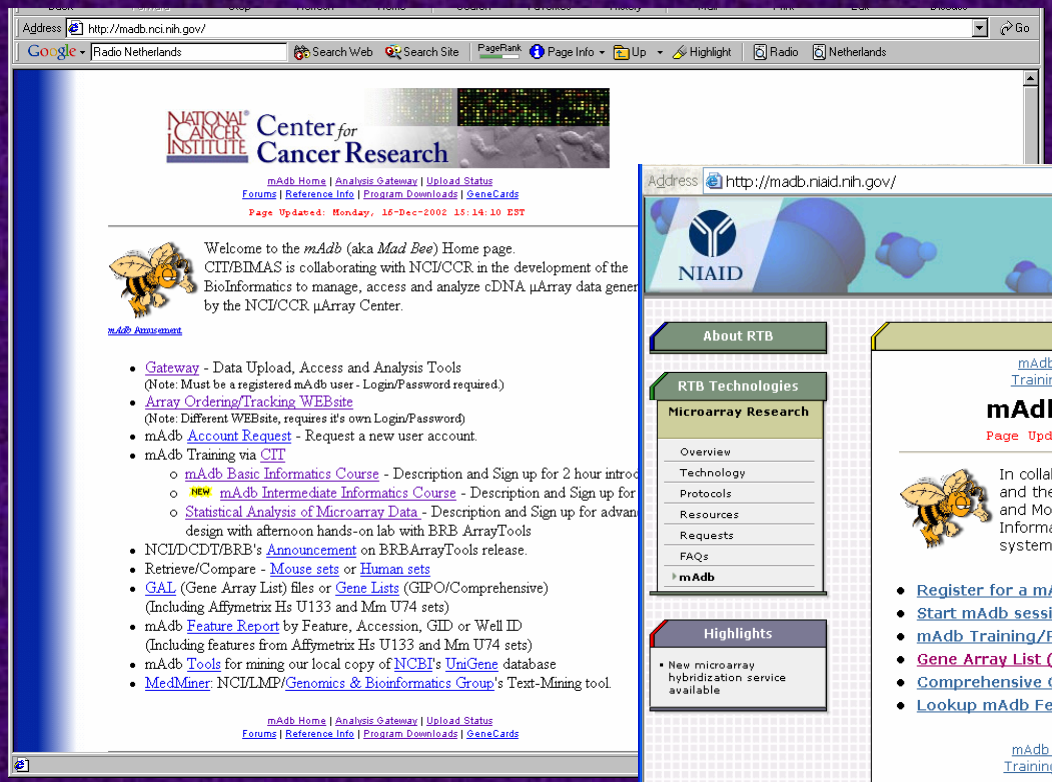
Goal:

- **Provide an integrated set of web-based analysis tools and a data management system for storing and analyzing cDNA/oligo/Affy Gene Expression data using open systems design, focusing on 2 color array slides.**
- **System currently supports spotted arrays routinely produced by the NCI, NIAID, and FDA Microarray Centers**
- **Currently support Axon GenePix, Perkin-Elmer QuantArray, and Arraysuite II / IP Lab image analysis software (Yidong Chen, NHGRI) for two-color, "Pat Brown-type" spotted arrays**
- **Affymetrix now available after a consultation to learn needed parameters – limited number of chips supported right now (mouse, human, rat)**

mAdb Home Page URLs

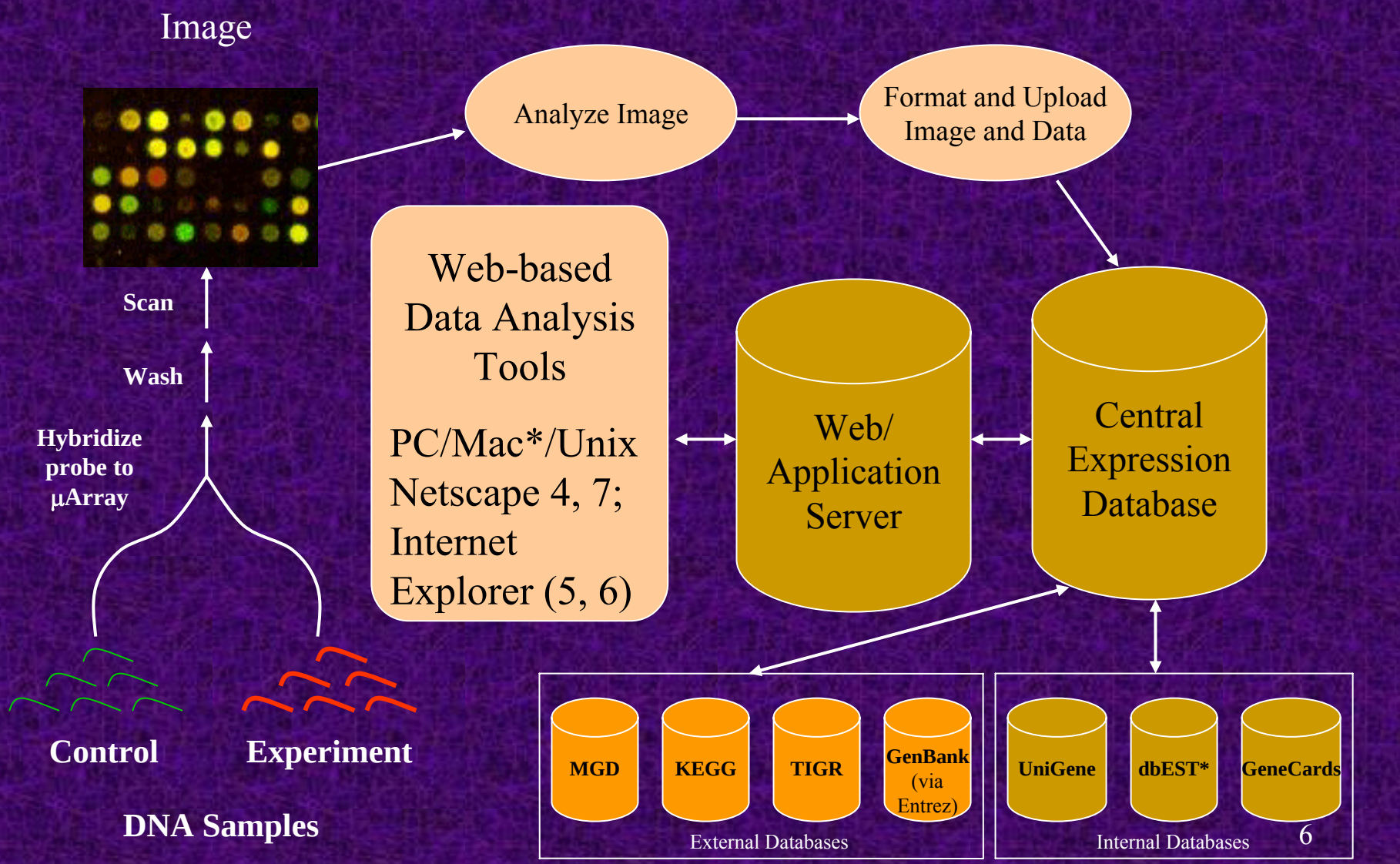
http://madb.nci.nih.gov

http://madb.niaid.nih.gov



For support, please e-mail: madb_support@bimas.cit.nih.gov

Architecture for μArray Informatics



Current *mAdb* Statistics

- 42,222 Arrays uploaded since Feb. 2000 – now average ~1020 per month uploaded over last year
- Roughly ¾ billion cDNA expression measurement points
- 1,180 registered users (NIH and collaborators)
- Among the largest collections of microarray data in the world, although data sharing is determined by <u>each</u> investigator – <u>no one</u> has access to all the data
- MIAME compliance coming <u>very soon</u>
- Can help you get data into public repositories – GEO (NCBI), ArrayExpress (EBI)

NIAID

CIT

mAdb System Features

- Gene Discovery
  - Outlier detection – row retrieval tools
  - Scatter plots
  - *Ad hoc* keyword queries
  - Multiple array viewer
- Class Comparison
  - t-test; Wilcoxon; ANOVA; Kruskal-Wallis
- Class Prediction
  - PAM classifier
- Class Discovery (unsupervised)
  - Clustering – Hierarchical, K-means, SOMs
  - Multidimensional Scaling
  - Principal Components Analysis
- Pathway summary – GO, KEGG, BioCarta
- Boolean comparison of data

Class #412 - Analyzing Microarray Data using the mAdb System

Live Demo

Home Page Notes

- Special Notices to Users
- Analysis Gateway link
- Account Requests link
- Array Tracker link – N.B. separate login & password!
- Training signup links
- GAL/GIPO links

mAdb GAL files

Current (2002) NCI Production Gene Array List Files (GAL files) (blocks x columns x rows)

- NEW Earlier NCI production printings
- Custom printings
- NIAID printings
- NEW FDA printings
- Mini-lymphochip GAL files (restricted to registered users)

Human Array Sets	
GAL File	**Array Sets**
Hs-UniGEM2-v2px-32Bx18Cx18R.gal Generated Tuesday, 21-May-2002 09:21:59 EDT Note: Also use for 2.1px, 2.3px, 2.4px, 2.5px, 2.6px, 5.0px See below for special **3.5px** gal file NEW See below for special **4.0px** gal file NEW See below for special **4.1px** gal file NEW See below for special **4.2px** gal file	Hs-UniGEM2-v2.4p1 Hs-UniGEM2-v2.4p2 Hs-UniGEM2-v2.4p3 Hs-UniGEM2-v2.4p4 Hs-UniGEM2-v2.4p5 Hs-UniGEM2-v2.4p8 Hs-UniGEM2-v2.4p9 Hs-UniGEM2-v2.5p3 Hs-UniGEM2-v2.5p4 Hs-UniGEM2-v2.5p5 Hs-UniGEM2-v2.5p6 Hs-UniGEM2-v2.5p7 Hs-UniGEM2-v2.5p11 Hs-UniGEM2-v2.6p2 Hs-UniGEM2-v2.6p3 Hs-UniGEM2-v2.6p6 Hs-UniGEM2-v2.6p7 Hs-UniGEM2-v2.6p8 Hs-UniGEM2-v2.6p9 Hs-UniGEM2-v2.6p10 Hs-UniGEM2-v5.0p1 Hs-UniGEM2-v5.0p2 Hs-UniGEM2-v5.0p3 Hs-UniGEM2-v5.0p4 Hs-UniGEM2-v5.0p5 Hs-UniGEM2-v5.0p6 Hs-UniGEM2-v5.0p7 Hs-UniGEM2-v5.0p8 Hs-UniGEM2-v5.0p9 Hs-UniGEM2-v5.0p10 Hs-UniGEM2-v5.0p12 Hs-UniGEM2-v5.0p13 Hs-UniGEM2-v5.0p14 Hs-UniGEM2-v5.0p15 Hs-UniGEM2-v5.0p16 Hs-UniGEM2-v5.0p17 Hs-UniGEM2-v5.0p18
Hs-UniGEM2-v3.5px-32Bx19x17R.gal Generated Tuesday, 21-May-2002 09:33:10 EDT	Hs-UniGEM2-v3.5p1 Hs-UniGEM2-v3.5p2
Hs-UniGEM2-4.0px-32Bx18Cx18R.gal Generated Monday, 25-Nov-2002 15:03:35 EST	Hs-UniGEM2-v4.0p2 Hs-UniGEM2-v4.0p4 Hs-UniGEM2-v4.0p5 Hs-UniGEM2-v4.0p6 Hs-UniGEM2-v4.0p7 Hs-UniGEM2-v4.0p8 Hs-UniGEM2-v4.0p9 Hs-UniGEM2-v4.0p10 Hs-UniGEM2-v4.0p11
Hs-UniGEM2-4.1px-32Bx18Cx18R.gal Generated Monday, 25-Nov-2002 15:34:59 EST	Hs-UniGEM2-v4.1p1

- Shows the actual GAL (Gene Array list) files – link block, row, column to what DNA is spotted there
- One printset layout is usually used for many lots of slides
- Please e-mail mAdb support if you cannot find your GAL file listed

Application Program Downloads

mAdb Program Downloads

Page Updated: Friday, 15-Aug-2003 08:45:58 EDT

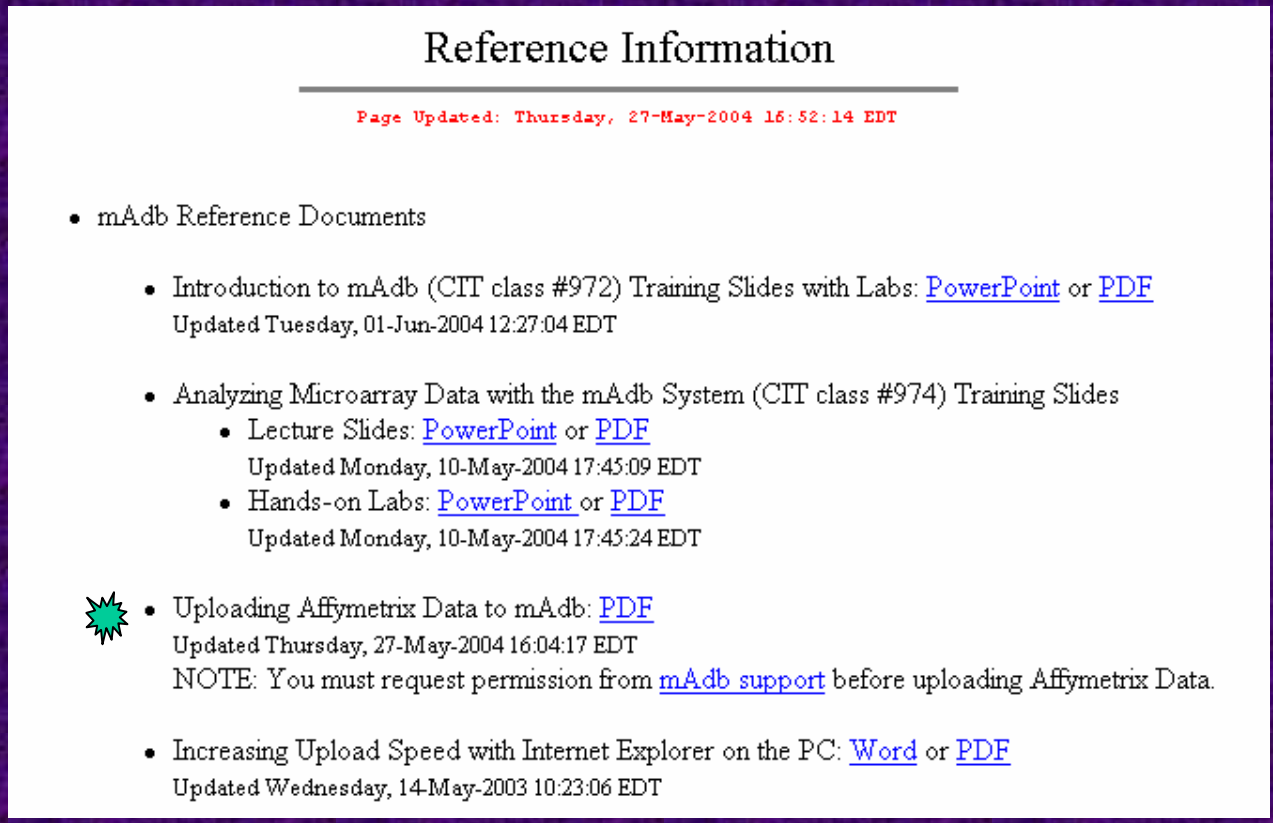
		Program	Description	Author	Version	Updated	Download	Manual
Axon Inc. Software This is commercial, licensed software and the GenePix application requires a "dongle" attached to the parallel port to run. The manual is accessible to all. **Axon's Web Site**		**GenePix Pro 5**	Fully integrated acquisition and analysis software for the GenePix 4000, 4100 & 4200. Download to a folder of your choice and then run to start the installation process.		5.0.1.13 History	8/15/2003 (Posted here 8/15/2003)	Download	Users Guide & Tutorial (PDF)
Axon Inc. Software This is commercial, licensed software and the GenePix application requires a "dongle" attached to the parallel port to run. The manual is accessible to all. **Axon's Web Site**		**GenePix Pro 4**	Fully integrated acquisition and analysis software for the GenePix 4000 & 4100. Download to a folder of your choice and then run to start the installation process.		4.0.1.17 History	(Posted here 3/12/2003)	Download	Manual Axon Scanner Manual (PDFs)
Axon Inc. Software This is commercial, licensed software and the GenePix application requires a "dongle" attached to the parallel port to run. The manual is accessible to all. **Axon's Web Site**		**GenePix Pro 3**	Fully integrated acquisition and analysis software for the GenePix 4000A. Download to a folder of your choice and then run to extract the installation files. Then run the extracted file setup.exe and follow installation instructions		3.0.6.89 History	(Posted here 02/18/2002)	Download	Manual Axon Scanner Manual (PDFs)
Stanford Genome Analysis Group Software It is available free of charge to academic and non-profit institutions. **Eisen Lab Download Site**	SA	**ScanAlyze**	Image Analysis (extracts data from fluorescence images of arrays)	Michael Eisen	2.44	11/15/99	Download	Manual (PDF)
		Cluster	Perform Hierarchical Clustering, Self-organizing Maps, k-Means Clustering, and More	Michael Eisen	2.11.01	7/10/2000 (Posted here 10/26/2000)	Download	Manual (PDF)
		TreeView	Graphical Viewing and Browsing of Cluster Results	Michael Eisen	1.5	04/2000 (Posted here	Download	
EASE: Expression Analysis Systematic Explorer Developed by the Laboratory of Immunopathogenesis and Bioinformatics, SAIC Frederick **EASE Web Site**		**EASE**	For finding "biological meaning" of gene lists via three functions: biological theme over-representation analysis, creation of annotation tables, and automated loading of genes into various online tools.	Doug Hosack	Revision history	Current version	Link to Download	Online help (Online)
MAExplorer Developed by and Available from LECB/FCRF/NCI. **MAExplorer Web Site**		**MAExplorer**	A Java data mining application for gene expression data using a variety of statistical, clustering, direct-manipulation graphical, spreadsheet and Web access methods.	Peter Lemkin	Revision History	Current version	Link to Download	Manual (Online) Use with mAdb data (PDF)

GenePix 5 is now supported, with important bug fix for spot images

Page accessible from NIH network only

Prefer GenePix updates obtained from this page – validated to work with mAdb

Reference Page



Reference Information

Page Updated: Thursday, 27-May-2004 16:52:14 EDT

- mAdb Reference Documents
  - Introduction to mAdb (CIT class #972) Training Slides with Labs: PowerPoint or PDF
    Updated Tuesday, 01-Jun-2004 12:27:04 EDT
  - Analyzing Microarray Data with the mAdb System (CIT class #974) Training Slides
    - Lecture Slides: PowerPoint or PDF
      Updated Monday, 10-May-2004 17:45:09 EDT
    - Hands-on Labs: PowerPoint or PDF
      Updated Monday, 10-May-2004 17:45:24 EDT
  - Uploading Affymetrix Data to mAdb: PDF
    Updated Thursday, 27-May-2004 16:04:17 EDT
    NOTE: You must request permission from mAdb support before uploading Affymetrix Data.
  - Increasing Upload Speed with Internet Explorer on the PC: Word or PDF
    Updated Wednesday, 14-May-2003 10:23:06 EDT

- Also links to protocols
- GenePix manuals
- N.B. Still must request Affy privileges be turned on for your account

II. Managing Projects and Uploading Arrays

Data Upload

- Login to Analysis Gateway page
  - change password if first-time user (case sensitive)
- Create project - logical organization for arrays
- Grant project access to others (if desired)
- Return to gateway and select project
- Select **type** of array for project
  - Spotted OR
  - Affymetrix (need to request permission via e-mail for first usage so we can give you needed parameters)

mAdb Gateway- link for Project Creation & Management

mAdb Gateway

NEW Create/Manage Projects link under Management Tools below. From there you can Create, Edit and Delete (empty projects) projects as well as Manage Access to projects.

Choose one or more Projects, select a Tool and Continue
or access previously extracted data located in **ncidemo**'s:
Temporary or Permanent area

Projects:

- XX guest - Time Course Demo Set #1
- XX guest - Time Course Demo Set #2
- XX guest - Repeats and Reciprocal Retests Demo Set #3
- XX guest - Multiple Types Demo Set #4
- AU ncidemo - my project
- AU ncidemo - Oligo and cDNA

Note: Tools marked with "*" only support selection of one project

Tool: Project Summaries Report

Continue

Uploading Links

- Upload Array data
- Status of Uploads
- Upload Identifier lists
- Manage Identifier lists

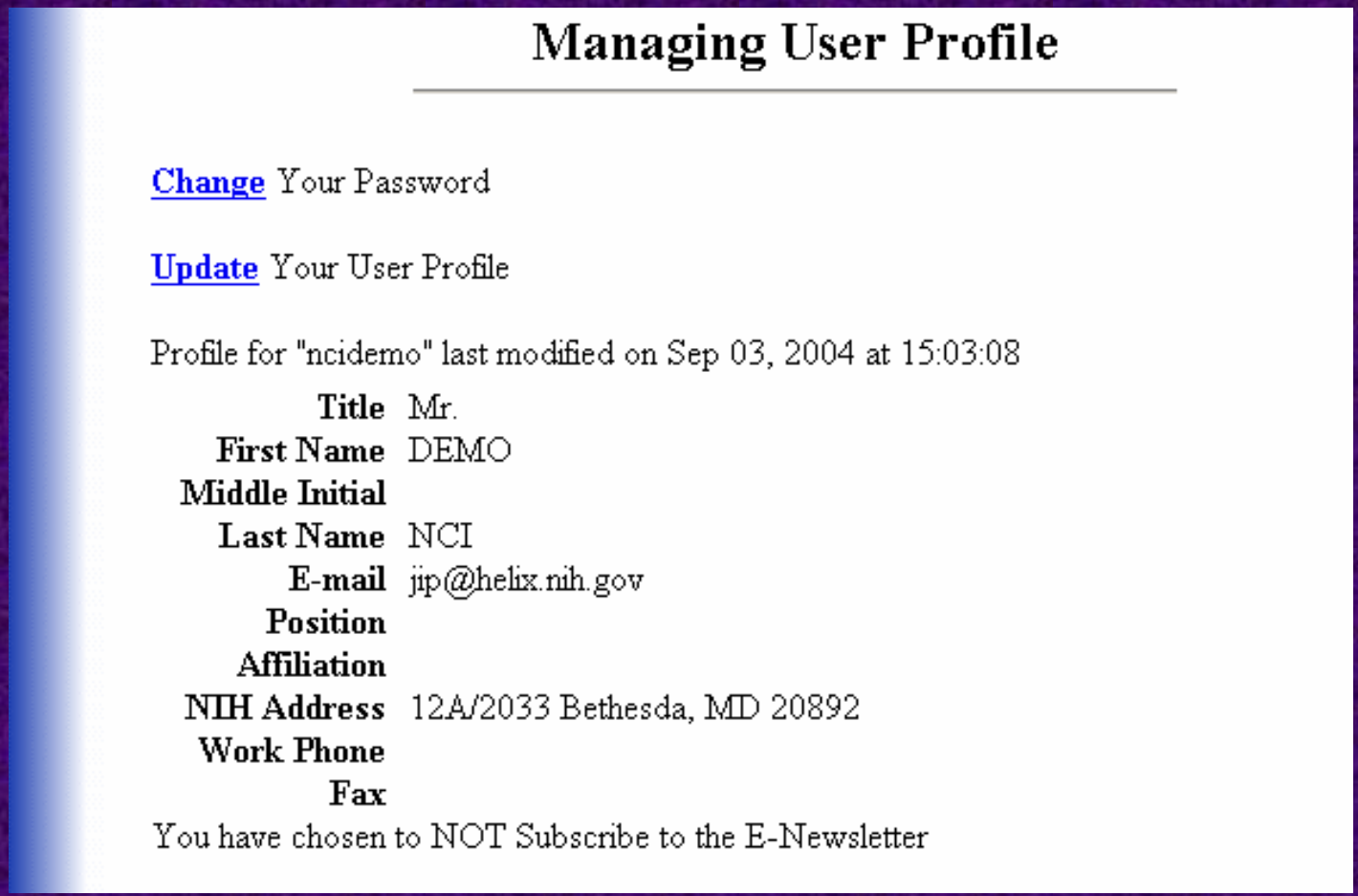
Management Tools

- Create/Manage Projects
- Manage User Profile

Access Training/Public Datasets

Access Additional Public Datasets

User Profile Management



Managing User Profile

Change Your Password

Update Your User Profile

Profile for "ncidemo" last modified on Sep 03, 2004 at 15:03:08

Title	Mr.
First Name	DEMO
Middle Initial	
Last Name	NCI
E-mail	jip@helix.nih.gov
Position	
Affiliation	
NIH Address	12A/2033 Bethesda, MD 20892
Work Phone	
Fax	

You have chosen to NOT Subscribe to the E-Newsletter

Managing Projects

Managing Projects

Create New Project

Shown below are existing Projects for which "ncidemo" is an administrator.
Projects are ordered first by the Creator and then by the Creation Date
In the Access List, **Bold** indicates a user with administrative access

Management Options
mAdb ID# 160 created by "ncidemo" on Jun 26, 2000 at 15:47:00 contains 10 Arrays

Project Title: my project
Description: Description by jip. Altered @1:00pm on 8/31/2004 and altered again by "easaki" on 9/1/2004
Comments: Comments by jip. Altered 8/31/2004
Access List: **easaki**, **jmgreene**, jpowell, **ncidemo**

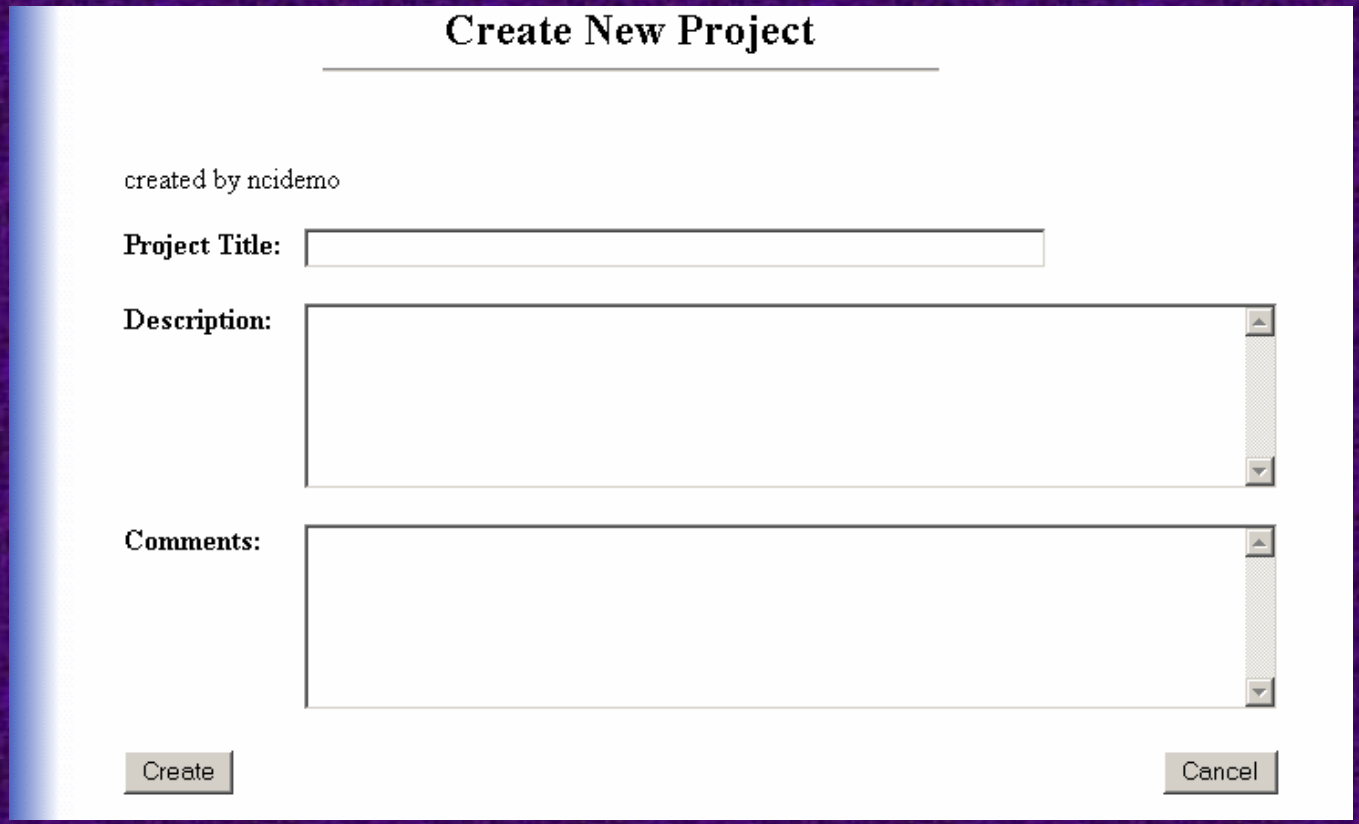
Management Options
mAdb ID# 1195 created by "ncidemo" on May 30, 2002 at 13:53:50 contains 10 Arrays

Project Title: Oligo and cDNA
Description: mixture of oligo and cDNA arrays
Comments: for IM class
Access List: easaki, **ncidemo**

Management Options
mAdb ID# 2874 created by "ncidemo" on Jun 24, 2004 at 13:27:42 contains no arrays

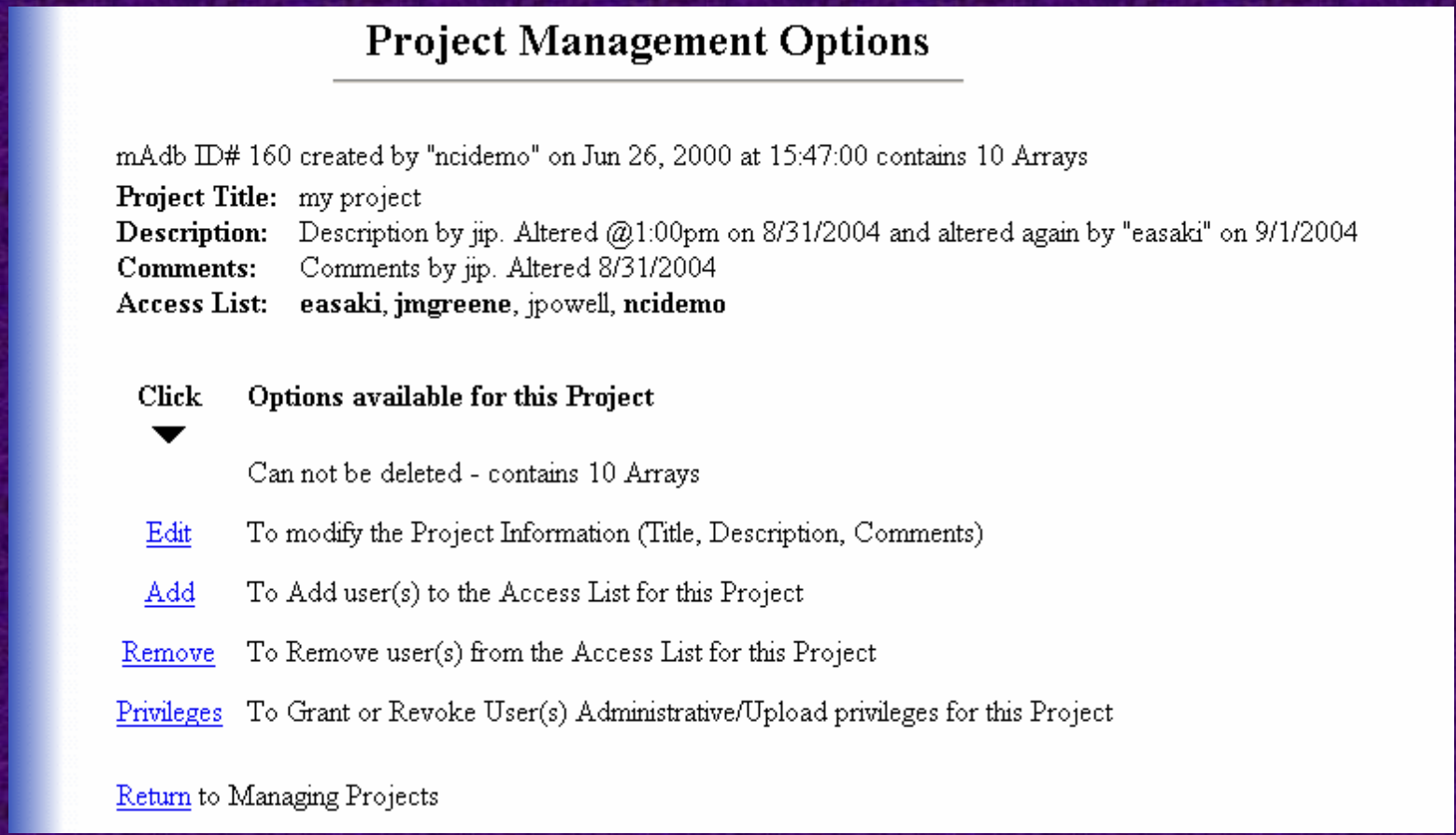
Project Title: Drug abcd
Description: jkas;ldkjflk
Comments: ;lkjljk;alsdjfsklk
Access List: easaki, **ncidemo**

Create New Project



- **A Project is a logical grouping of your arrays**

Project Management Options



Project Management Options

mAdb ID# 160 created by "ncidemo" on Jun 26, 2000 at 15:47:00 contains 10 Arrays

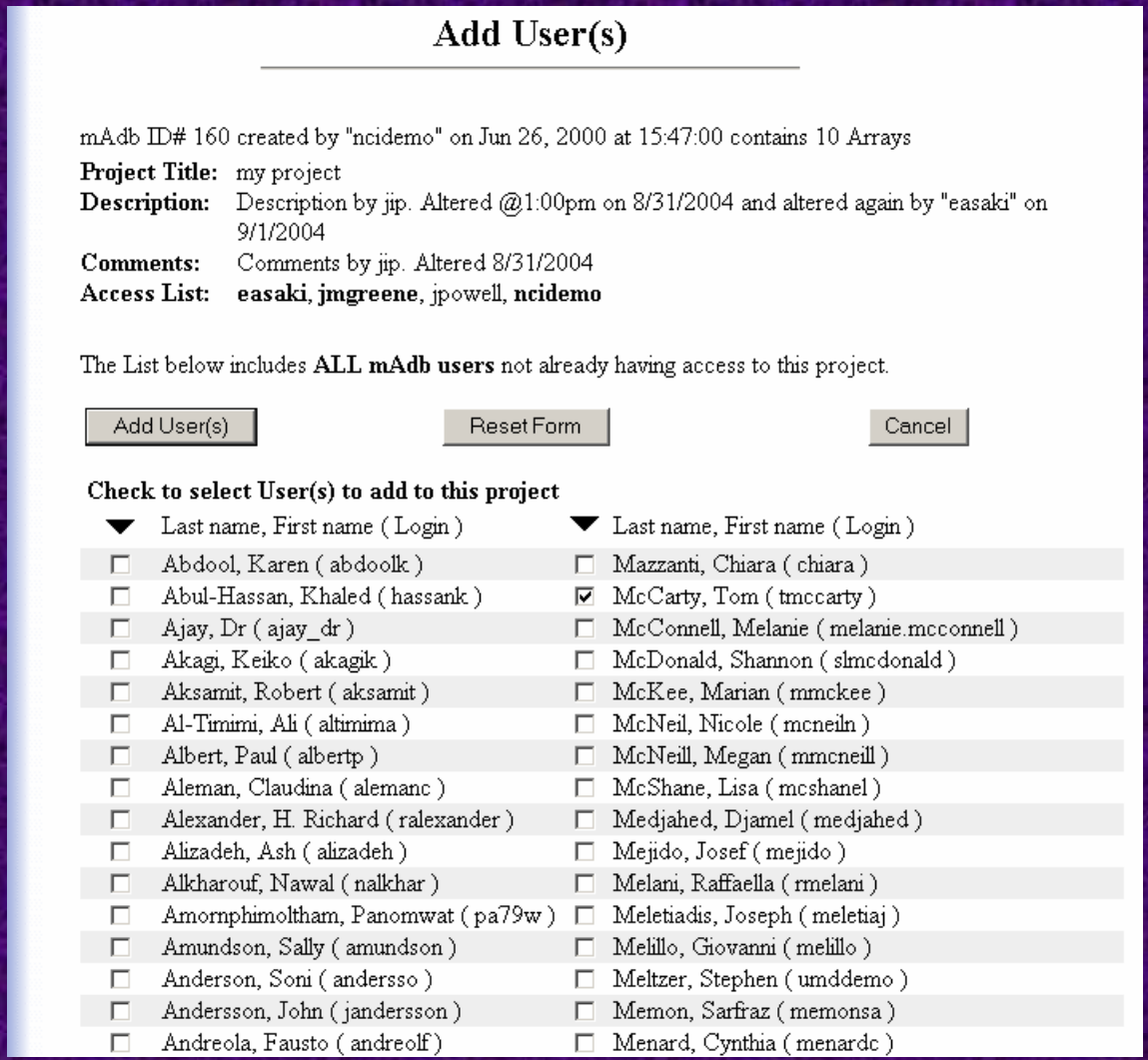
Project Title: my project
Description: Description by jip. Altered @1:00pm on 8/31/2004 and altered again by "easaki" on 9/1/2004
Comments: Comments by jip. Altered 8/31/2004
Access List: **easaki**, **jmgreene**, jpowell, **ncidemo**

Click ▼	Options available for this Project
	Can not be deleted - contains 10 Arrays
Edit	To modify the Project Information (Title, Description, Comments)
Add	To Add user(s) to the Access List for this Project
Remove	To Remove user(s) from the Access List for this Project
Privileges	To Grant or Revoke User(s) Administrative/Upload privileges for this Project

Return to Managing Projects

Bold names on access list indicate administrative privileges for account

Project Access



Add User(s)

mAdb ID# 160 created by "ncidemo" on Jun 26, 2000 at 15:47:00 contains 10 Arrays

Project Title: my project

Description: Description by jip. Altered @1:00pm on 8/31/2004 and altered again by "easaki" on 9/1/2004

Comments: Comments by jip. Altered 8/31/2004

Access List: **easaki**, **jmgreene**, jpowell, **ncidemo**

The List below includes **ALL mAdb users** not already having access to this project.

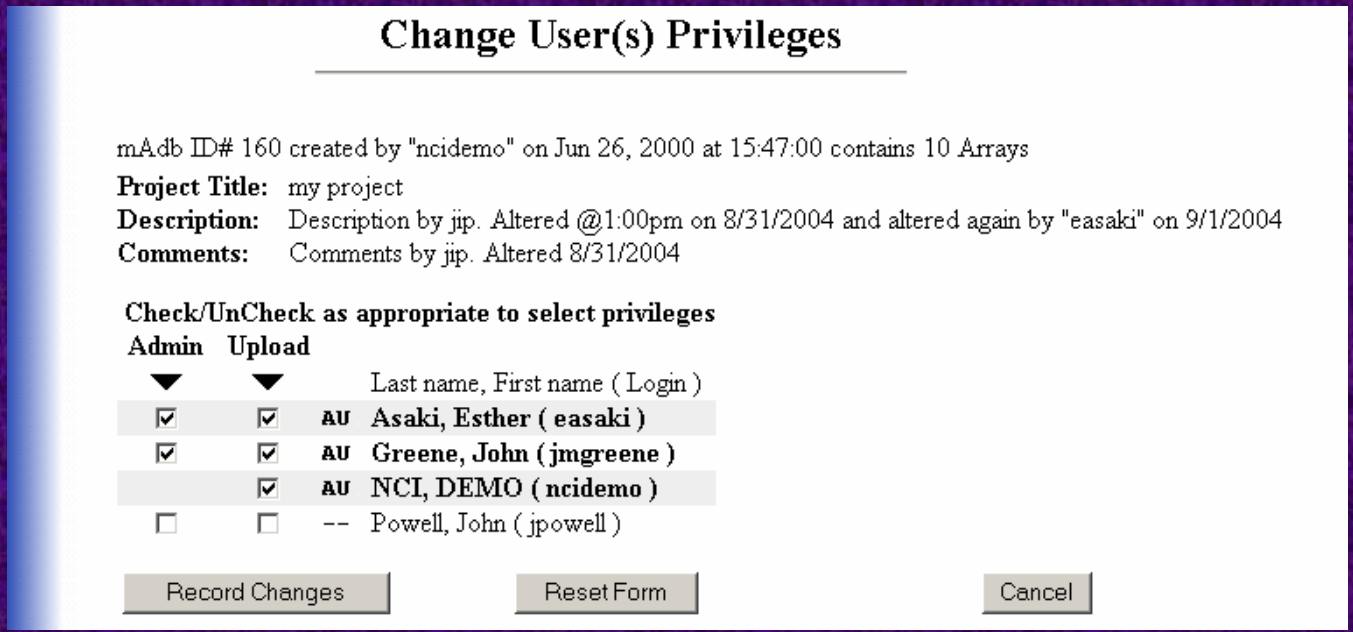
Add User(s) | Reset Form | Cancel

Check to select User(s) to add to this project

Last name, First name (Login)	Last name, First name (Login)
☐ Abdool, Karen (abdoolk)	☐ Mazzanti, Chiara (chiara)
☐ Abul-Hassan, Khaled (hassank)	☑ McCarty, Tom (tmccarty)
☐ Ajay, Dr (ajay_dr)	☐ McConnell, Melanie (melanie.mcconnell)
☐ Akagi, Keiko (akagik)	☐ McDonald, Shannon (slmcdonald)
☐ Aksamit, Robert (aksamit)	☐ McKee, Marian (mmckee)
☐ Al-Timimi, Ali (altimima)	☐ McNeil, Nicole (mcneiln)
☐ Albert, Paul (albertp)	☐ McNeill, Megan (mmcneill)
☐ Aleman, Claudina (alemanc)	☐ McShane, Lisa (mcshanel)
☐ Alexander, H. Richard (ralexander)	☐ Medjahed, Djamel (medjahed)
☐ Alizadeh, Ash (alizadeh)	☐ Mejido, Josef (mejido)
☐ Alkharouf, Nawal (nalkhar)	☐ Melani, Raffaella (rmelani)
☐ Amornphimoltham, Panomwat (pa79w)	☐ Meletiadis, Joseph (meletiaj)
☐ Amundson, Sally (amundson)	☐ Melillo, Giovanni (melillo)
☐ Anderson, Soni (andersso)	☐ Meltzer, Stephen (umddemo)
☐ Andersson, John (jandersson)	☐ Memon, Sarfraz (memonsa)
☐ Andreola, Fausto (andreolf)	☐ Menard, Cynthia (menardc)

Adding a user allows that mAdb account holder to view your arrays in a project and work with the data to create filtered datasets

User Privileges



Change User(s) Privileges

mAdb ID# 160 created by "ncidemo" on Jun 26, 2000 at 15:47:00 contains 10 Arrays

Project Title: my project
Description: Description by jip. Altered @1:00pm on 8/31/2004 and altered again by "easaki" on 9/1/2004
Comments: Comments by jip. Altered 8/31/2004

Check/UnCheck as appropriate to select privileges

Admin	Upload		Last name, First name (Login)
☑	☑	AU	**Asaki, Esther (easaki)**
☑	☑	AU	**Greene, John (jmgreene)**
	☑	AU	**NCI, DEMO (ncidemo)**
☐	☐	--	Powell, John (jpowell)

Record Changes | Reset Form | Cancel

Privileges allow user to:

- Upload Arrays
- Administer access to arrays and edit project, array, and dataset descriptions

mAdb Tool Gateway- link for uploading

mAdb Gateway

NEW Create/Manage Projects link under Management Tools below. From there you can Create, Edit and Delete (empty projects) projects as well as Manage Access to projects.

Choose one or more Projects, select a Tool and Continue
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Projects:

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- XX guest - Multiple Types Demo Set #4
- AU ncidemo - my project
- AU ncidemo - Oligo and cDNA

Note: Tools marked with "*" only support selection of one project

Tool: Project Summaries Report

Continue

Uploading Links

- Upload Array data
- Status of Uploads
- Upload Identifier lists
- Manage Identifier lists

Management Tools

- Create/Manage Projects
- Manage User Profile

Access Training/Public Datasets

Access Additional Public Datasets

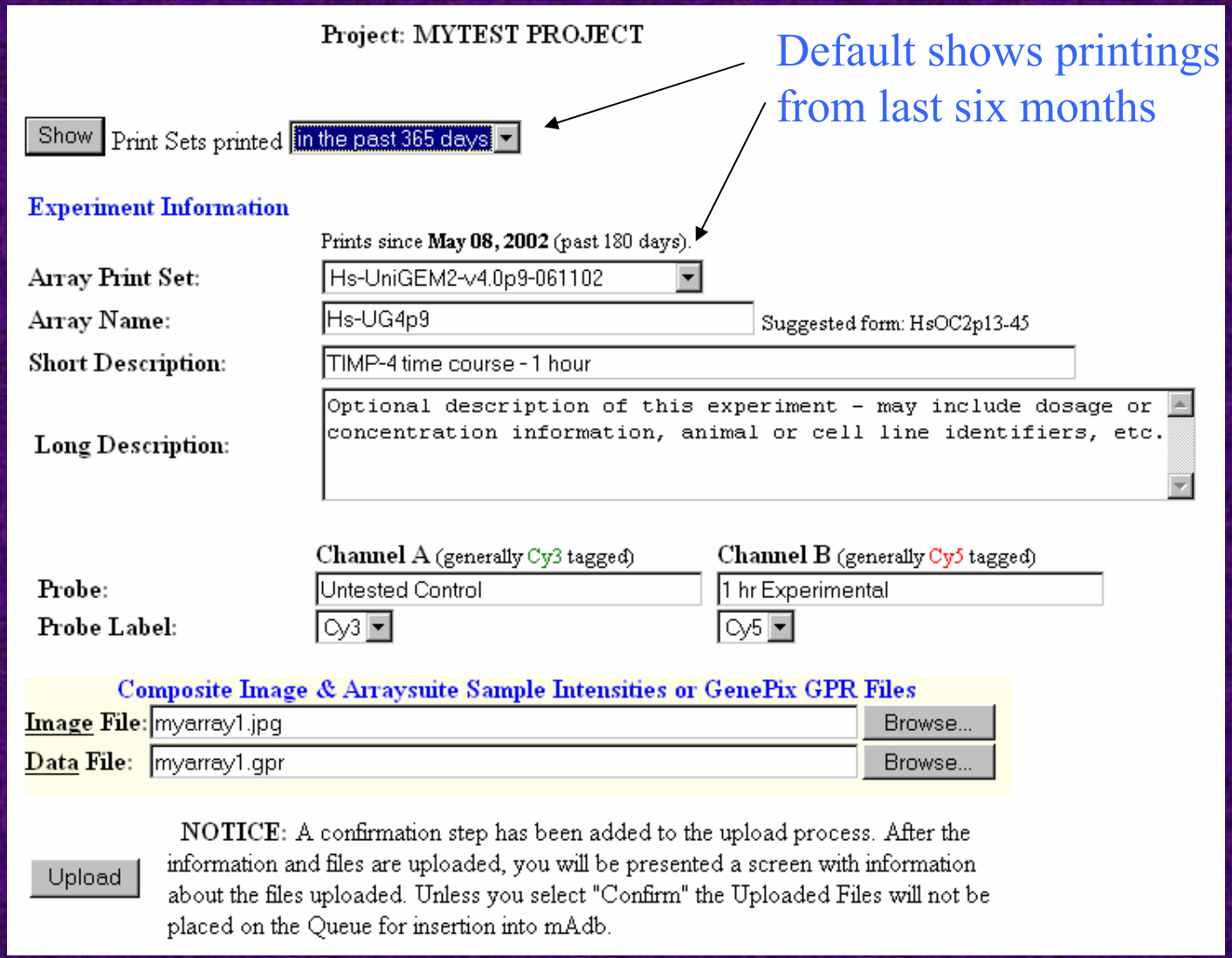
Spotted Array Data Upload

- Fill in experimental info for each array
  - Pick Print Set
  - Select image file of array
  - Select data file for array
- Submit and confirm upload
- Check status page to display progress
- Close browser when finished (for security)

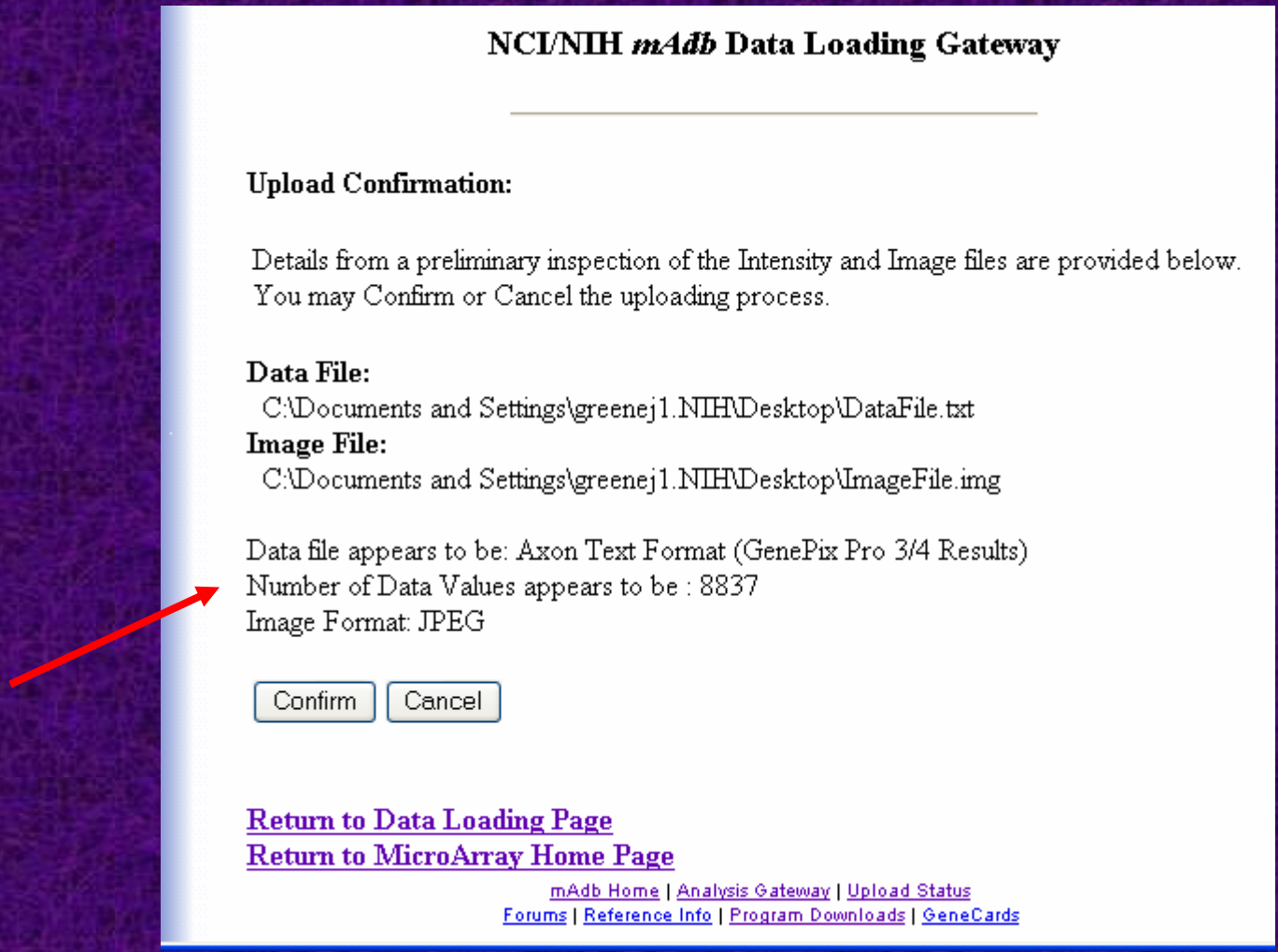
Affymetrix Data Upload

- Select:
  - Data File (Metrics - .txt file)
  - CEL file
- Fill in Experiment data
- Submit and confirm upload
- Check status page to display progress
- Close browser when finished (for security)

Uploading Spotted Arrays

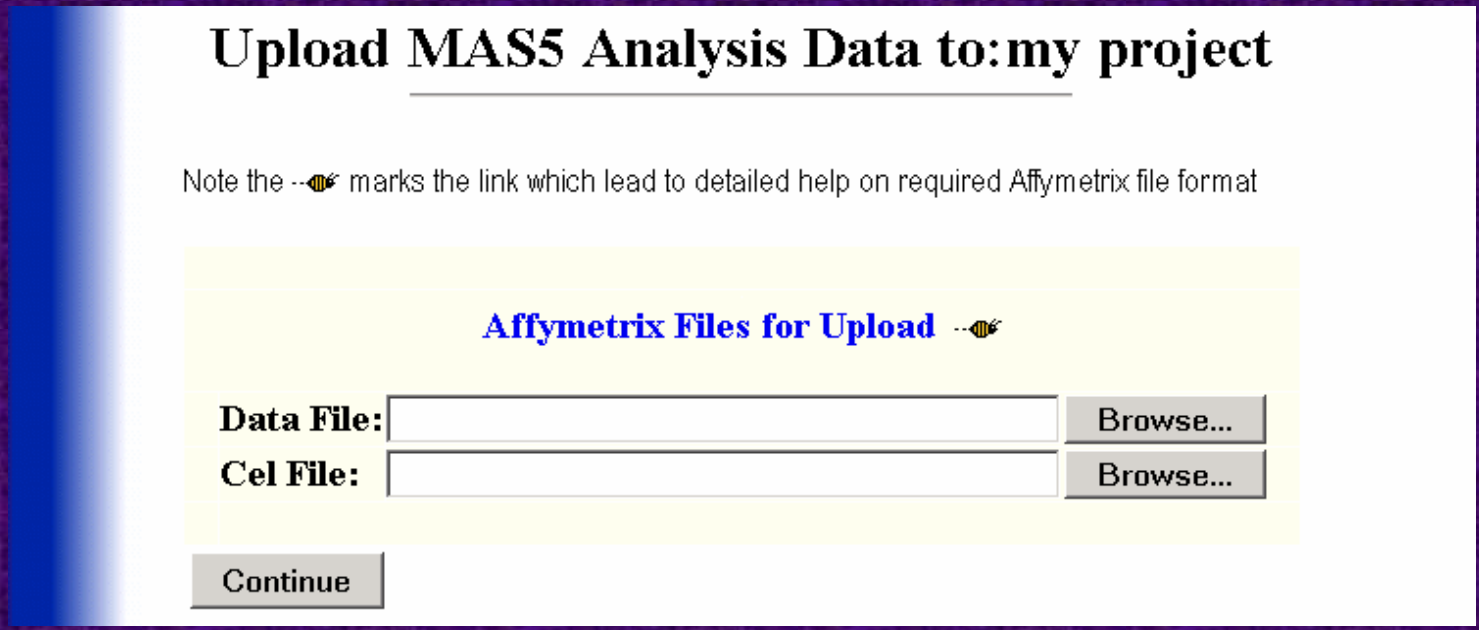


Confirming Upload



You should check that the image and file type appear correct and that the file line count is roughly equal to the number of spots on the array

Adding Affy Arrays



- Browse to Metrics (*.txt) file for the Data File box
- Browse to the corresponding .CEL file in second box

Adding Affy Arrays

Confirm Affymetrix Genechip Data

Experiment Information
You have uploaded Absolute Analysis data for a Human Genome Array U95A genechip.
The Data have not been scaled in your analysis.
Please check/complete the information on this page. Click the Confirm button to complete the upload process or use the Cancel button to abort and start again.

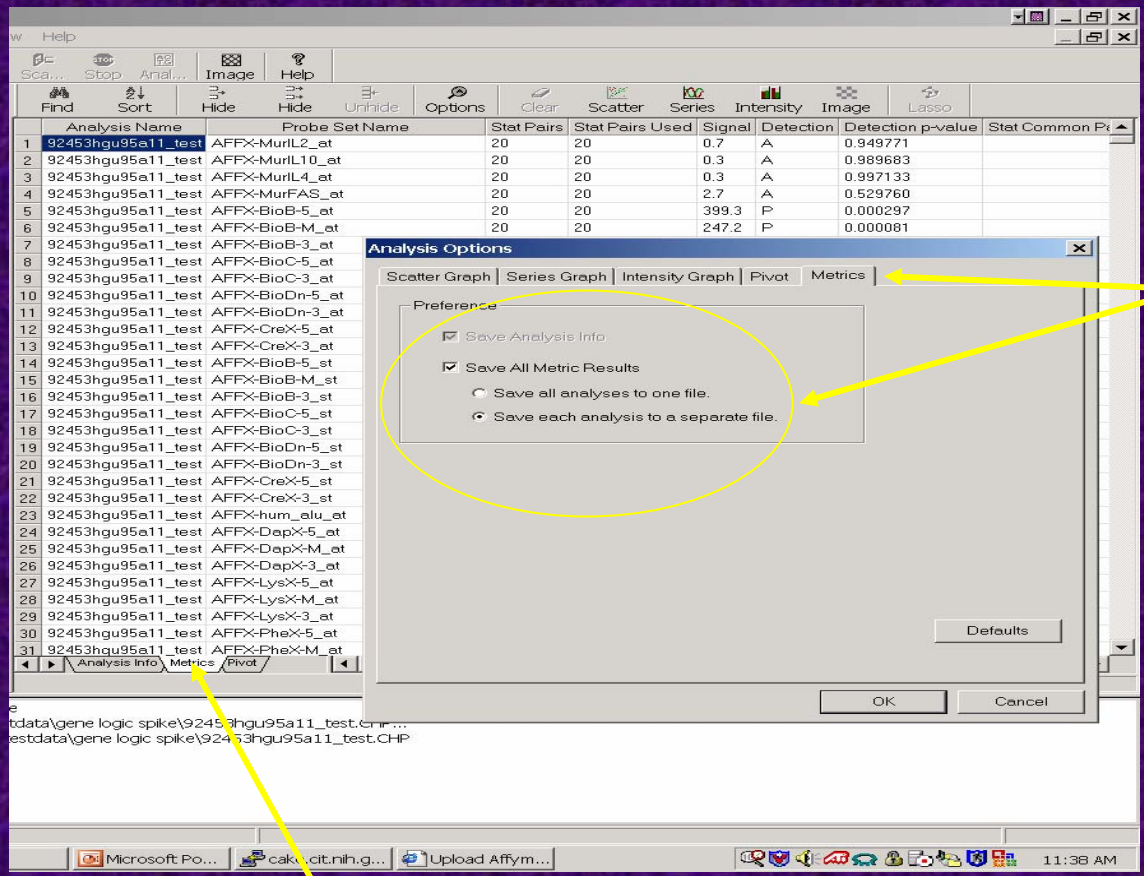
Uploaded Data File: C:\GeneChip\TESTDATA\Gene Logic Spike\92453hgu95a11_test.txt
Uploaded CEL File: C:\GeneChip\TESTDATA\Gene Logic Spike\92454hgu95a11.cel

Fields labled with ** are mandatoray.

Array Print Set:	U95A
Array Name: **	92453hgu95a11_test
Sample Type:	
Sample Description:	
Comments:	

Confirm Cancel

Affymetrix – CHP file

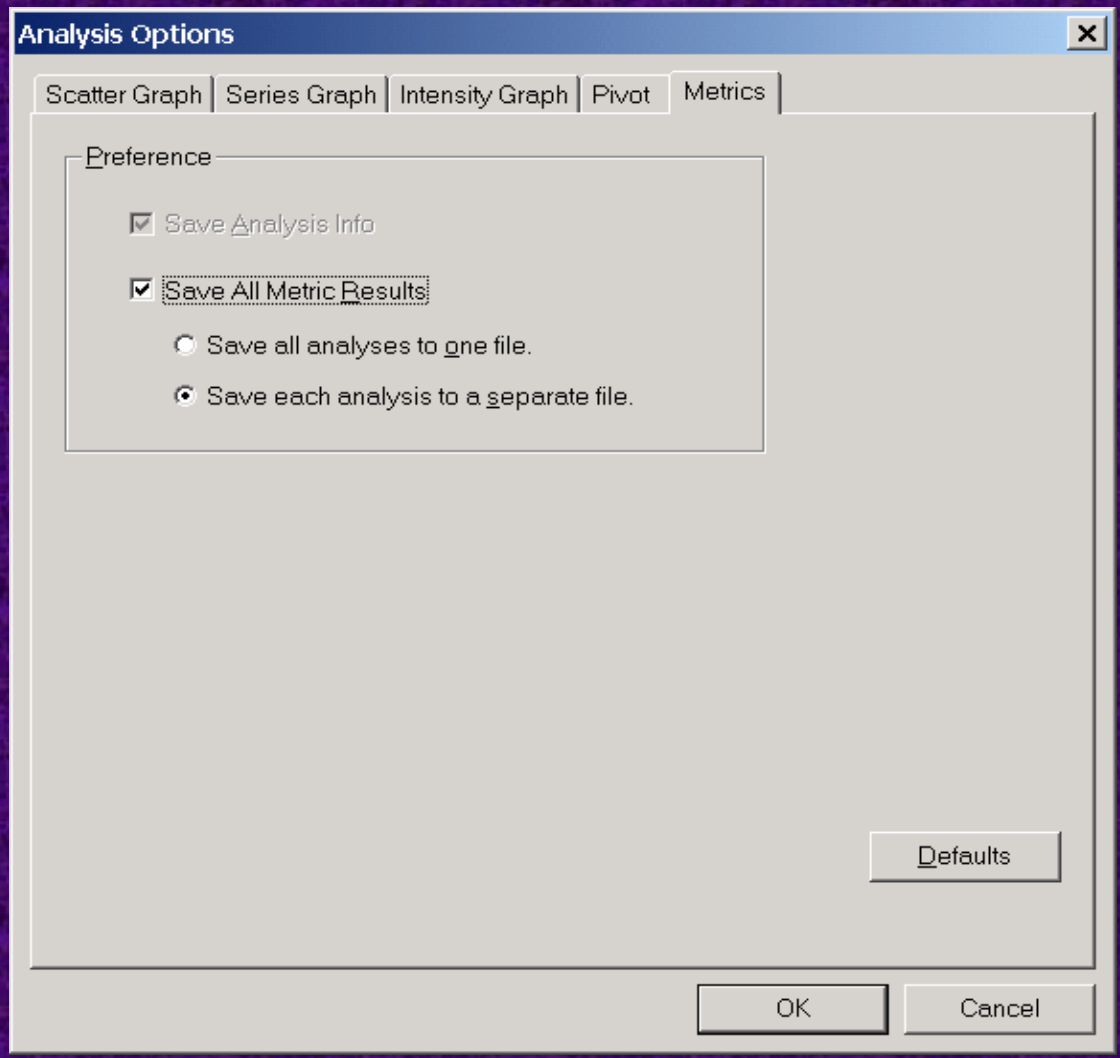


Set Metrics options:

- Save all Metric Results
- Save each analysis to a separate file

Select Metrics tab before saving

Affymetrix – CHP file Metrics options



Upload Status

- Shows your arrays and totals for all users
- Two step process:
  - Data is parsed and entered into Sybase db
  - Image is processed and stored
- You can work with data without waiting for image processing to finish

mAdb WEB Upload Status Report

Status Updated: Tue Sep 28 10:54:29 EDT 2004
(This page refreshes every 10 minutes)

Other mAdb WEB Upload Reports:
Graphical summary by month (past 12 months) or by day (past 90 days)
Details of arrays queued for processing
Details of arrays uploaded within the past 24 hours, 7 days, 30 days or all

mAdb login	Arrays	Status
ncidemo	0	Queued for/or loading into mAdb
Total all Users	0	Queued for/or loading into mAdb
ncidemo	0	Loaded; Queued for/or Image processing
Total all Users	0	Loaded; Queued for/or Image processing

Activity for the past 30 days

ncidemo	0	Processing completed
Total all Users	1037	Processing completed (332 Affymetrix, 705 Spotted)
ncidemo	0	Canceled, UnConfirmed, Bad Files/Rejected Submissions;
Total all Users	44	Canceled, UnConfirmed, Bad Files/Rejected Submissions

GenePix Analysis Notes

- Download correct GAL file from mAdb
- Carefully grid each block
- Allow program to “Find spots” and adjust spot size
- Set option to “Analyze absent spots”
- Adjust JPEG for desired contrast/brightness
- Analyze spots

Spotted Array Uploading Notes

- Include the slide number scratched on the slide as part of the Array Name, which will act as a unique identifier
- If array print was printed more than 6 months ago, extend time frame using the pulldown menu and press “Show” button
- When uploading to a new project, array prints from all species will be displayed
- When uploading to a project with arrays, only array prints using the same species in the project will be displayed.

Common Spotted Array Errors

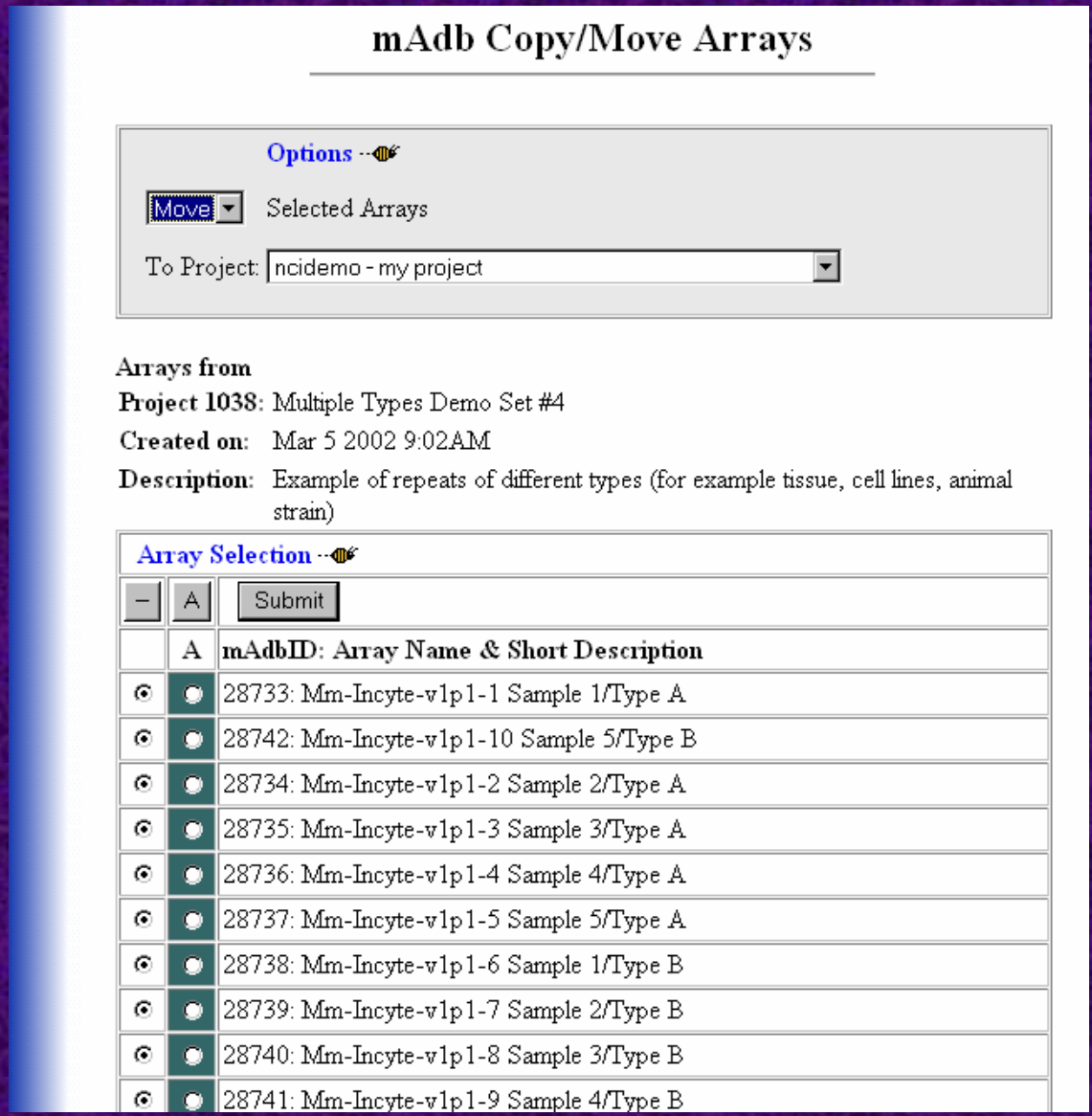
- Common Upload Errors
  - Choosing wrong print set
  - Loading GAL file, Excel file, or Set Up file in place of GenePix data (.gpr) file
  - Loading multi-image TIFF file instead of composite, single image JPEG or PICT file
- Common GenePix Errors
  - Setting incorrect option for “Analyze Absent Feature” (box should be checked) – results in truncated blocks
  - Deleted blocks
  - Improper gridding

Affymetrix Analysis Notes

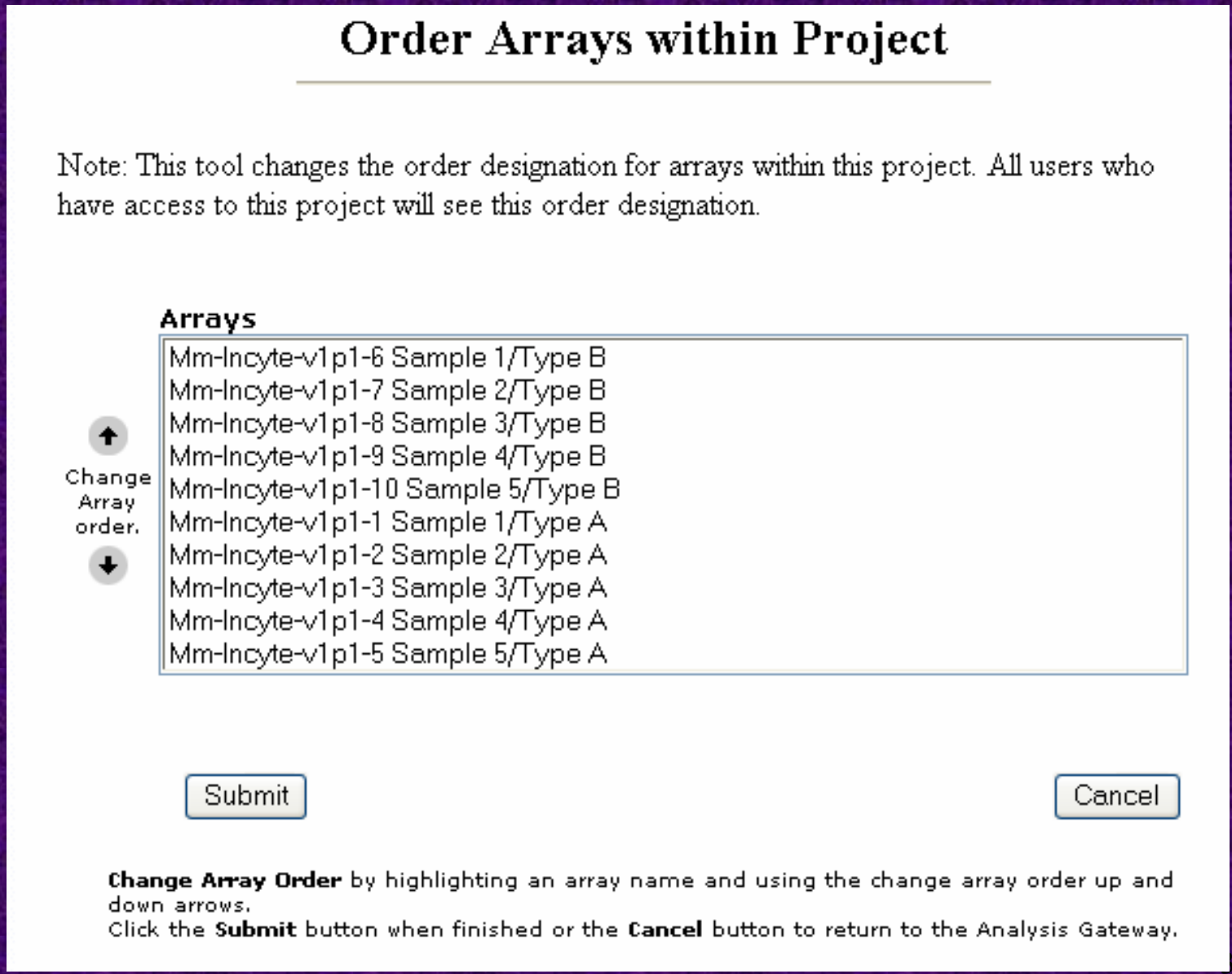
- Run chip through fluidics station to get CEL file
- Analyze CEL file (usually scale all spots to 500)
- With CHP file open, set analysis options on metrics tab as:
  - "Save Analysis Info"
  - "Save each analysis to a separate file"
- Click on Metric tab
- Save file as …. Xxxx.txt
- Note: If uploading comparison data, then upload absolute baseline data first.

Ability to copy or move arrays between projects

- Need administrative access to both projects
- Create a "trash" project to "delete" unwanted arrays



Ability to re-order arrays within a project



III. Initial Array Quality Analysis

- Signal definition
- Normalization
- Use of log base 2
- Project Summary Report
- Comprehensive Graphical Quality Report

mAdb Definitions

- Signal - refers to background corrected values (i.e.Target Intensity - Background Intensity).
- Defaults:
  - MEAN Intensity – MEDIAN background (for GenePix)
  - MEAN Intensity – MEAN background (for ArraySuite)
- Normalization factor – initially calculated so that the median overall ratio (Cy5 Signal/ Cy3 Signal) is adjusted to 1.0 (linear; 0.0, log base 2) for each array. Spots with an extremely low signal are excluded from this calculation.

Need for Normalization of Ratios

- Unequal incorporation of labels (green Cy3 incorporates better than red Cy5)
- Unequal amounts of samples
- Unequal PMT voltage settings

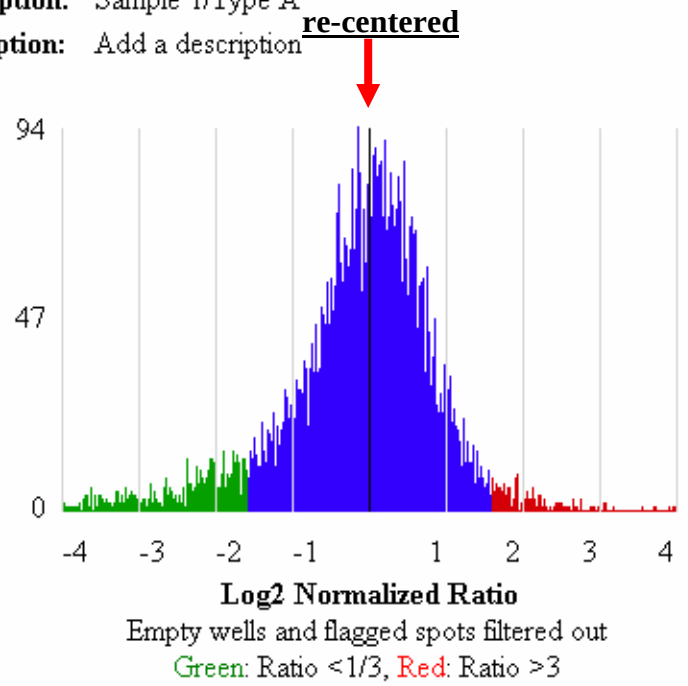
Whenever possible, use ratios converted to log base 2

- Why? Because it makes variation of ratios more independent of absolute magnitude
- Evens out highly skewed graphs, giving a more realistic sense of variation – upregulated genes graph from 1 to ∞ ; downregulated genes graph crammed between 0 and 1
- Easier interpretation – negative numbers are downregulated genes; positive numbers are upregulated genes

mAdb Array Histogram

Comprehensive Graphical Report (Be Patient!)

Array: Mm-Incyte-v1p1-1
Short Description: Sample 1/Type A
Long Description: Add a description



Empty wells and flagged spots filtered out
Green: Ratio <1/3, Red: Ratio >3

Mean Signal		Median Bkg		Sgl/Bkg		Not	Normal.
Ch A	Ch B	Ch A	Ch B	Ch A	Ch B	Found	Factor**
326	455	110	84	3.0	5.4	30%	0.617

Normalization factor is calculated and multiplied by all ratios to re-center array distribution around 1 (linear), equal to 0 in log base 2

Project Summary

mAdb Project Summaries 1.0

Retrieve Array Summaries formatted for MS-Excel

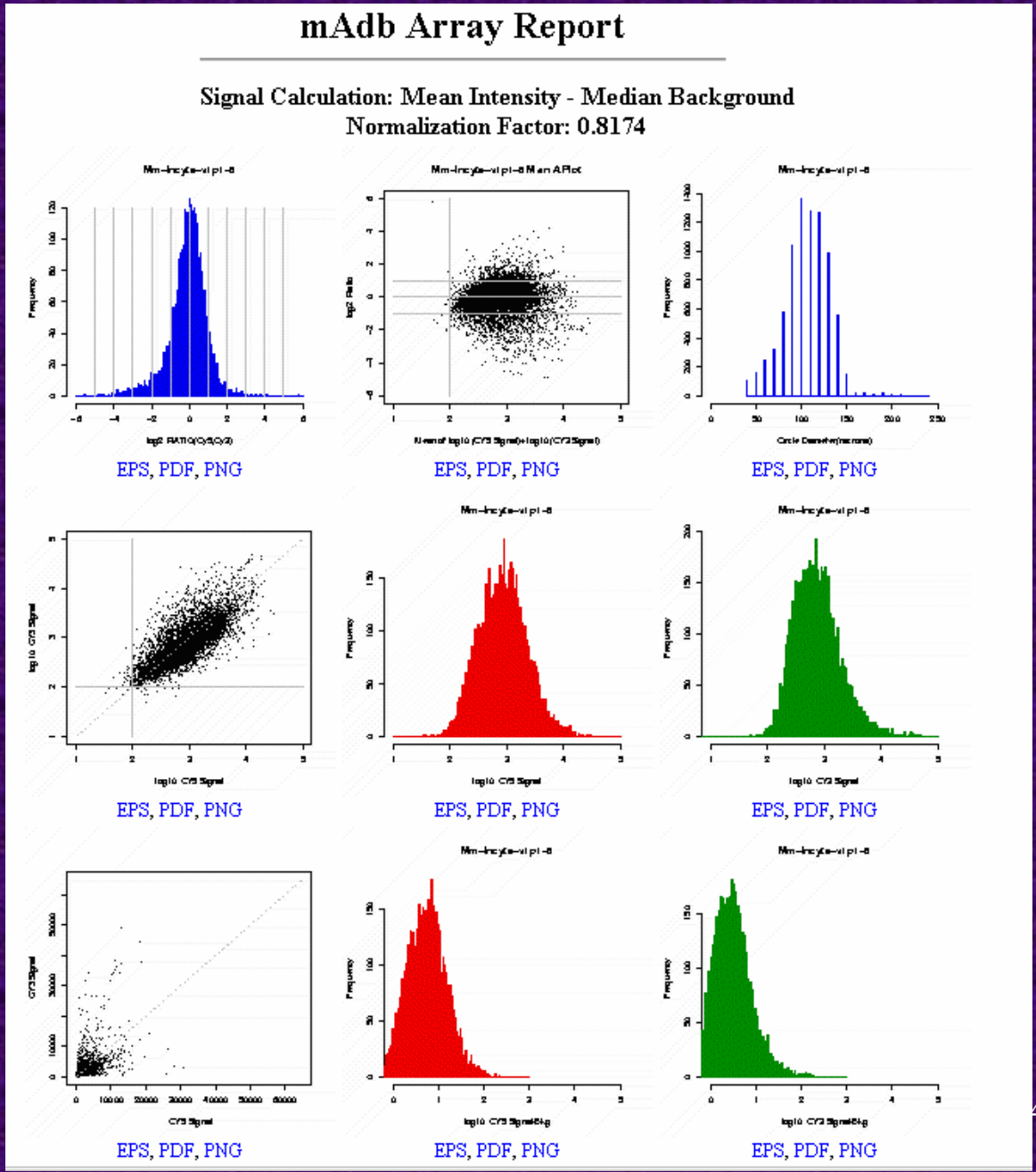
Edit **Project #1038:** Multiple Types Demo Set #4
Created on: Mar 05, 2002
Description: Example of repeats of different types (for example tissue, cell lines, animal strain)

		Summary Statistics								Array Information						
		Mean Signal Ch A	Mean Signal Ch B	Median Bkg Ch A	Median Bkg Ch B	Sgl/Bkg Ch A	Sgl/Bkg Ch B	% Found	Normal. Factor	mAdb ID	Uploaded	Array Print	Array	Probe A	Probe B	Short
Edit	1.	326	455	110	84	3.0	5.4	70%	0.626	28733	Mar 5 2002 9:07AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-1	Control	Sample 1	Sampl
Edit	2.	1677	2088	241	160	7.0	13.1	93%	0.769	28742	Mar 5 2002 9:24AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-10	Control	Sample 5/B	Sampl
Edit	3.	880	673	200	364	4.4	1.8	84%	1.055	28734	Mar 5 2002 9:10AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-2	Control	Sample 2	Sampl
Edit	4.	1056	1473	259	154	4.1	9.6	93%	0.658	28735	Mar 5 2002 9:11AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-3	Control	Sample 3	Sampl
Edit	5.	297	493	117	87	2.5	5.7	84%	0.542	28736	Mar 5 2002 9:13AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-4	Control	Sample 4	Sampl
Edit	6.	443	543	123	89	3.6	6.1	83%	0.708	28737	Mar 5 2002 9:15AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-5	Control	Sample 5	Sampl
Edit	7.	499	541	120	101	4.2	5.4	84%	0.858	28738	Mar 5 2002 9:17AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-6	Control	Sample 1/B	Sampl
Edit	8.	626	717	146	113	4.3	6.3	85%	0.890	28739	Mar 5 2002 9:21AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-7	Control	Sample 2/B	Sampl
Edit	9.	1280	1399	272	190	4.7	7.4	93%	0.830	28740	Mar 5 2002 9:22AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-8	Control	Sample 3/B	Sampl
Edit	10.	1113	1371	261	156	4.3	8.8	91%	0.779	28741	Mar 5 2002 9:23AM	Mm-Incyte-v1p1-090600	Mm-Incyte-v1p1-9	Control	Sample 4/B	Sampl

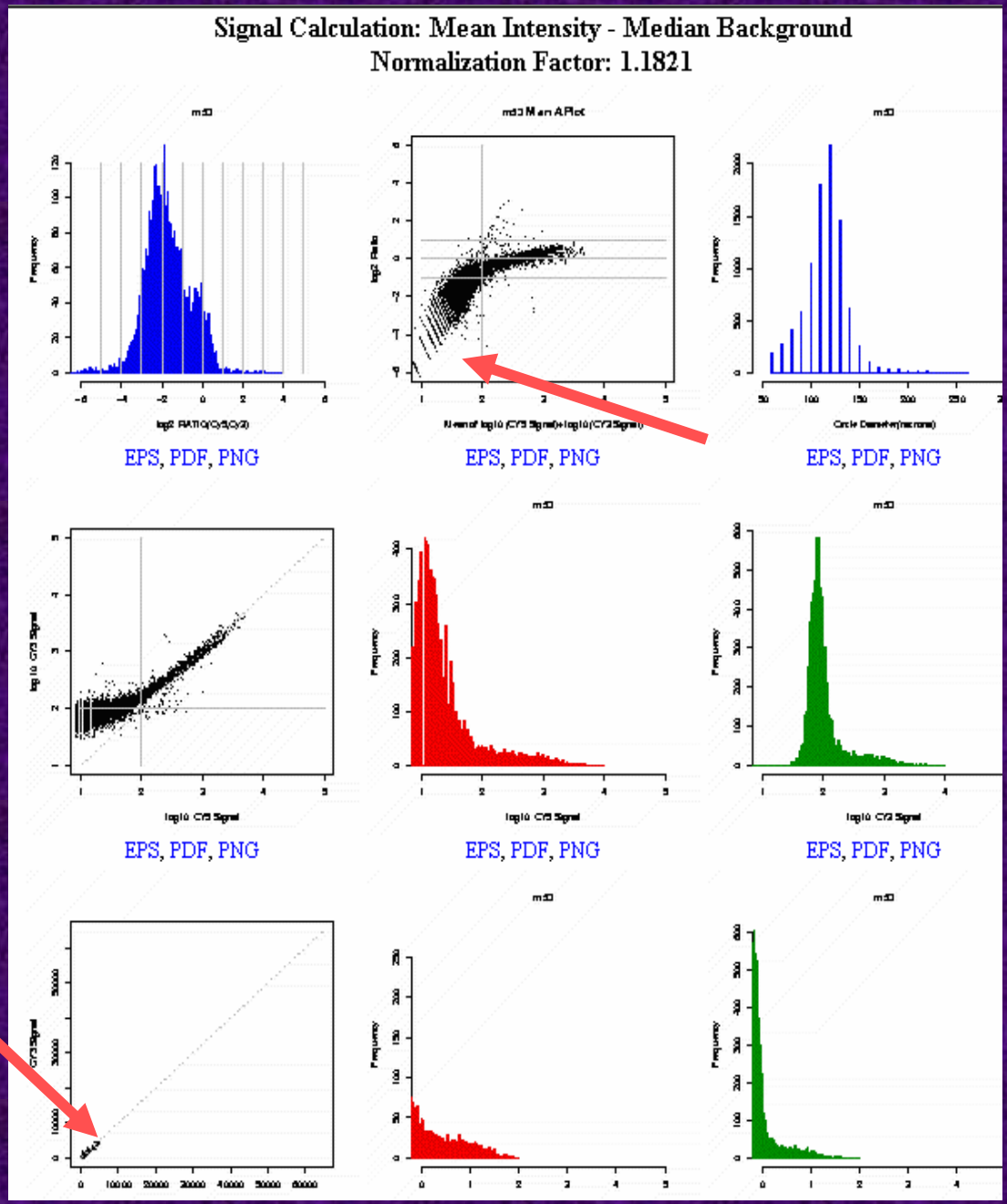
- Aid to QC – overall array statistics, links to histogram, array image
- If you have admin access to a project, can edit project and array descriptions from “Edit” links here

Comprehensive Graphical Quality Report

- Accessed from histogram display
- More QC parameters, including:
- M versus A plot
- spot size distribution
- log and linear plots of each channel
- signal intensity distribution
- signal/background distribution



Low Intensity Example



- M vs A plot – ratio distribution dependent upon signal strength; see a "tail" toward green spots
- Spot sizes small
- Overall signal strength very weak – not a good range of signals on Cy3/Cy5 linear plot
- Bulk of red signals less than 10
- FYI, max signal is 65,000

IV. Creating datasets and additional filtering

mAdb Analysis Paradigm:

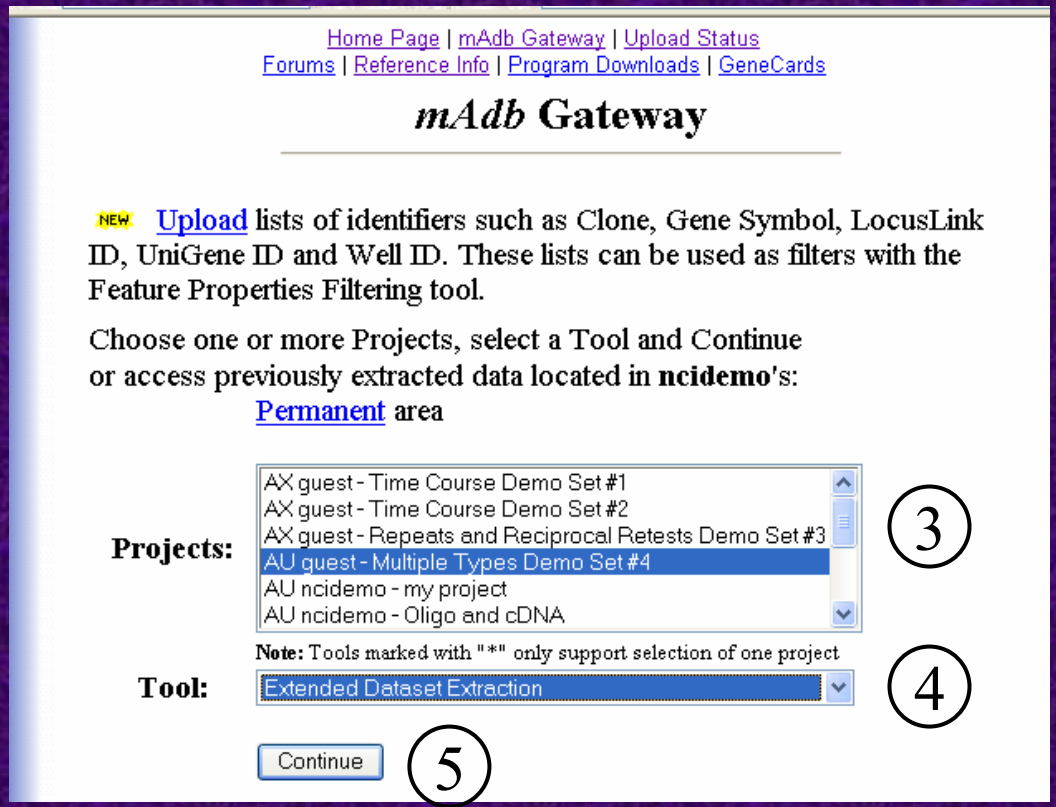
1. Create project; Upload arrays to that project
2. Quality control – Project Summary and Graphical Reports
3. Create a filtered dataset:
   - Extract rows from database
   - Filter spots on quality parameters (spot size, S/N, etc.)
   - Normalize, so different arrays can be compared
   - Align genes from different array layouts (based on well IDs)
4. Apply Data/Gene criteria filters, if desired, to create subset dataset(s)
5. Apply appropriate Analysis/Visualization Tools to the dataset(s)
6. Repeat Steps 3, 4, and 5 as desired
7. Interpret Datasets/Results

Lab 1 – Creating a filtered dataset

Goal: To use the Extended Extraction Tool to choose arrays from a project and filter on quality parameters.

Do NOT maximize the browser window, so multiple windows can be distinguished on the monitor.

Lab 1. Choosing Project and Extended Dataset Extraction Tool



1. Open a web browser and type the URL for the mAdb home page, **http://madb-training.cit.nih.gov** .

2. Click the first bullet on the home page, to access the **mAdb Gateway,** web page, shown at left. You will need to login the mAdb Gateway with the mAdb account as instructed.

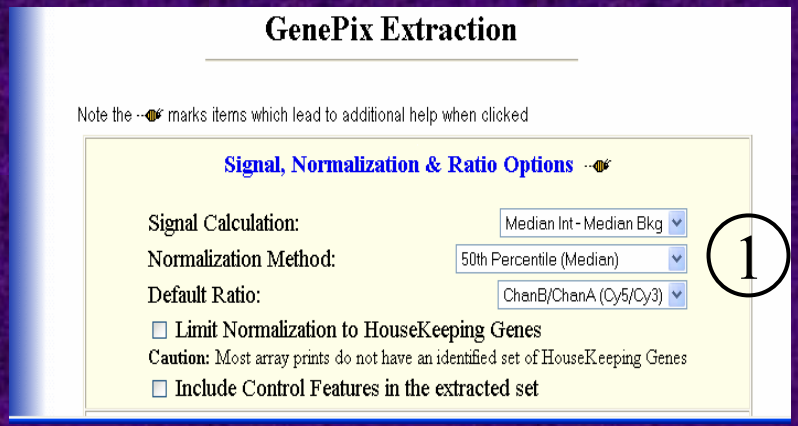
3. On the mAdb Gateway Web page, in the **Projects:** list, select the "**guest – Multiple Types Demo Set #4"** project

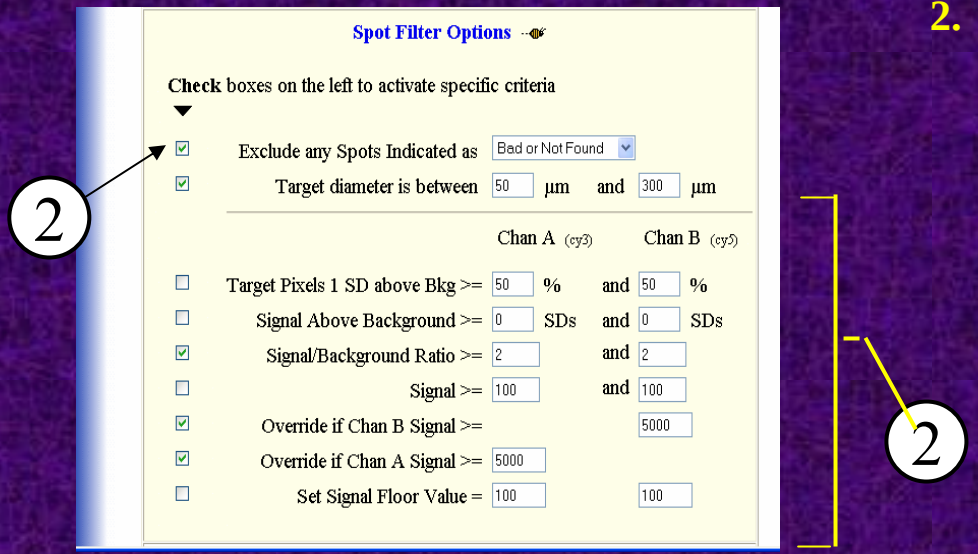
NOTE: You can select multiple projects by holding down the **Ctrl** key when you click on a project

4. On the **Tools:** menu just below, select "**Extended Dataset Extraction"**

5. Press the **Continue** button

Lab 1. Selecting Filtering Options



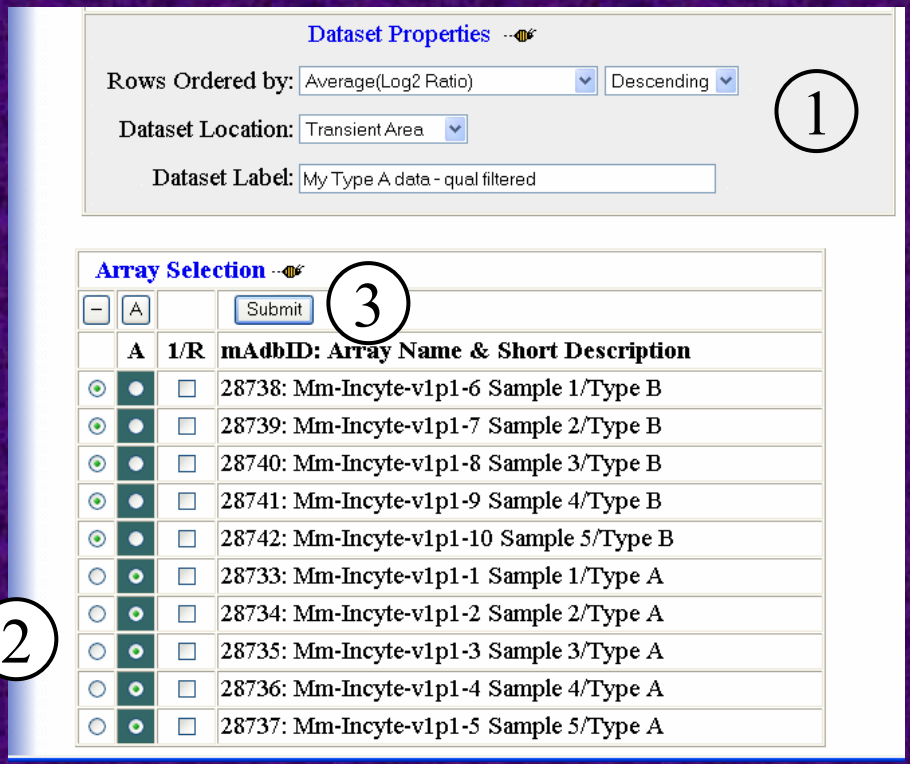


1. In the **Signal, Normalization, & Ratio Options** panel, choose **Signal Calculation:** Median Int – Median Bkg**, Normalization Method:** 50th Percentile (Median), and **Default Ratio:** ChanB/ChanA**.** Leave the checkboxes empty. Using this Normalization method, the output is re-normalized based on the spots which pass the filters.

2. In the **Spot Filter Options** panel, check the boxes on the left to activate the appropriate filter(s), and choose appropriate values by typing in numbers into the form elements to the right of each filter checkbox. For the purposes of this exercise, check:
   - Exclude any Spots indicated as **Bad or Not Found**
   - Target diameter is between **50** um and **300** um
   - Signal/background Ratio >= **2** and **2**
   - Override if Chan B Signal >= **5000**
   - Override if Chan A Signal >= **5000**

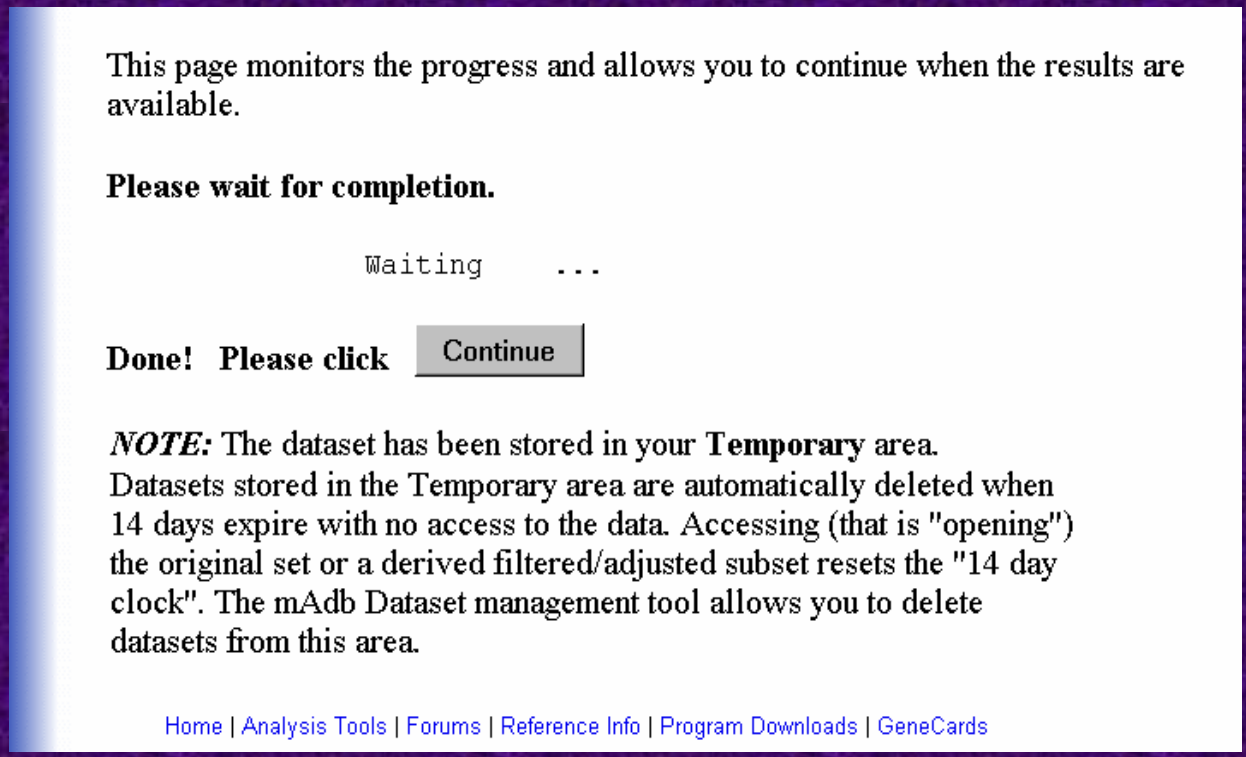
3. Go to next page of lab to choose arrays

Lab 1. Selecting Dataset Properties and Arrays



1. In the **Dataset Properties** panel, choose **Rows Ordered by: Average(Log2 Ratio)** and **Descending; Dataset Location: Transient Area,** and **Dataset Label:** "My Type A data – qual filtered" .
2. In the **Array Selection** panel, choose just the Type A arrays using the radio buttons under **A. N.B.** If a dye swap or reverse fluor, check the **1/R** box to take the reciprocal value of the ratio for direct comparison.
3. Press **Submit**

Lab 1. Waiting for Data Extraction …



Intermediate screen which monitors the data extraction process. When the creation of the working dataset is complete, the user can continue to the Data Display page.

Extended Tool: Signal, Normalization & Ratio Options:

- **Signal Calculation**

  Mean Intensity – Median Background

  Median Intensity – Median Background

- **Normalization**

  - None

  - 50th Percentile (Median)

  Applied to extracted spots (spot filtered)

  All spots or only Housekeeping spots (on limited prints)

  - Pre-calculated 50th percentile (uses all data)

  - Lowess non-linear normalization – in beta testing

- **Default Ratio**

  Chan B/Chan A (CY5/CY3),

  but for reverse fluor can choose Chan A/Chan B (CY3/CY5)

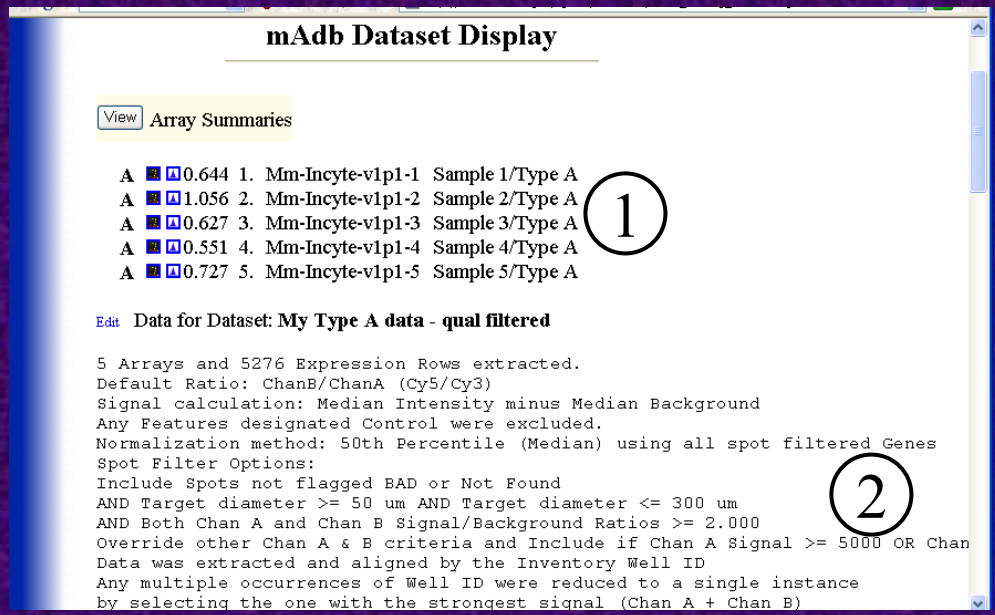
Spot Filter Options:

- **Important - Check box to Activate!**
- **Exclude any Spots Flagged as *Bad Or Not Found, Bad***
- **Target diameter is between *default of 50 and 300 microns***
- **Target Pixels 1 Standard Deviation above background >= *N %***
- **Signal above background >= *N SDs (standard deviations)***
- **Signal/Background Ratio >= *N***
- **Signal >= N *(raw signal intensities)***
- **Override bracketed criteria (in yellow above) if Chan B and /or A Signal >= *N (default is 5000)***

Signal Floor

- When one channel has a very low signal and the other has a moderate or high signal, the resulting ratio value could be misleading (i.e. very high/low)
- To adjust such a highly skewed ratio, mAdb allows the user to set a floor (e.g. 100) for signals below a threshold

Lab 1. Main mAdb Dataset Display – Part 1

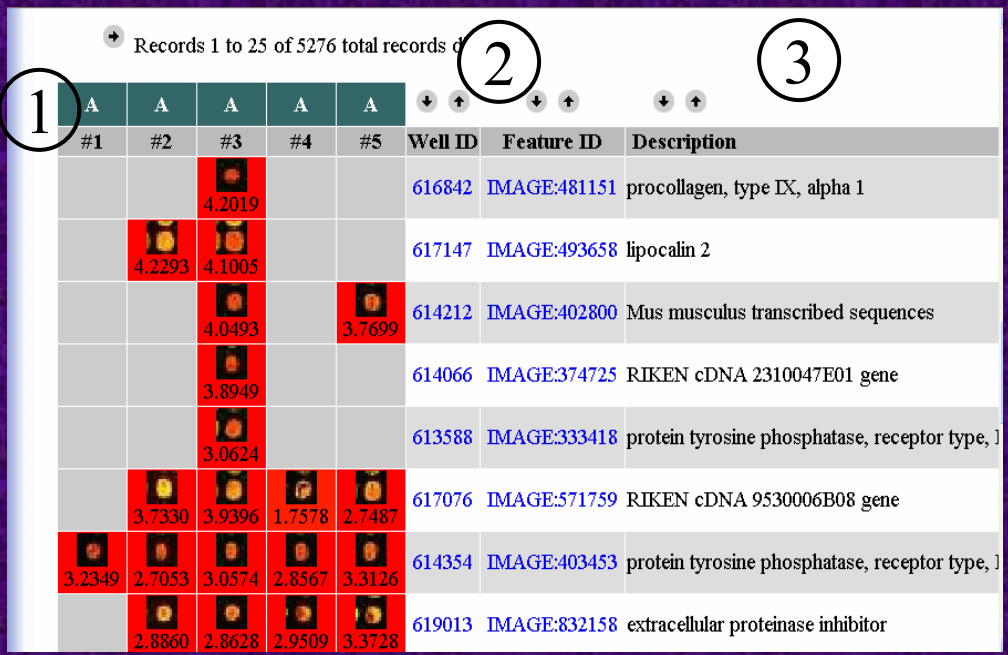


1. The listing at the top shows the array group, a link to the array image, a link to a histogram display, the re-calculated normalization factor (based on those spots which passed the quality filters), the array name, and the short description for all of the chosen arrays to be filtered

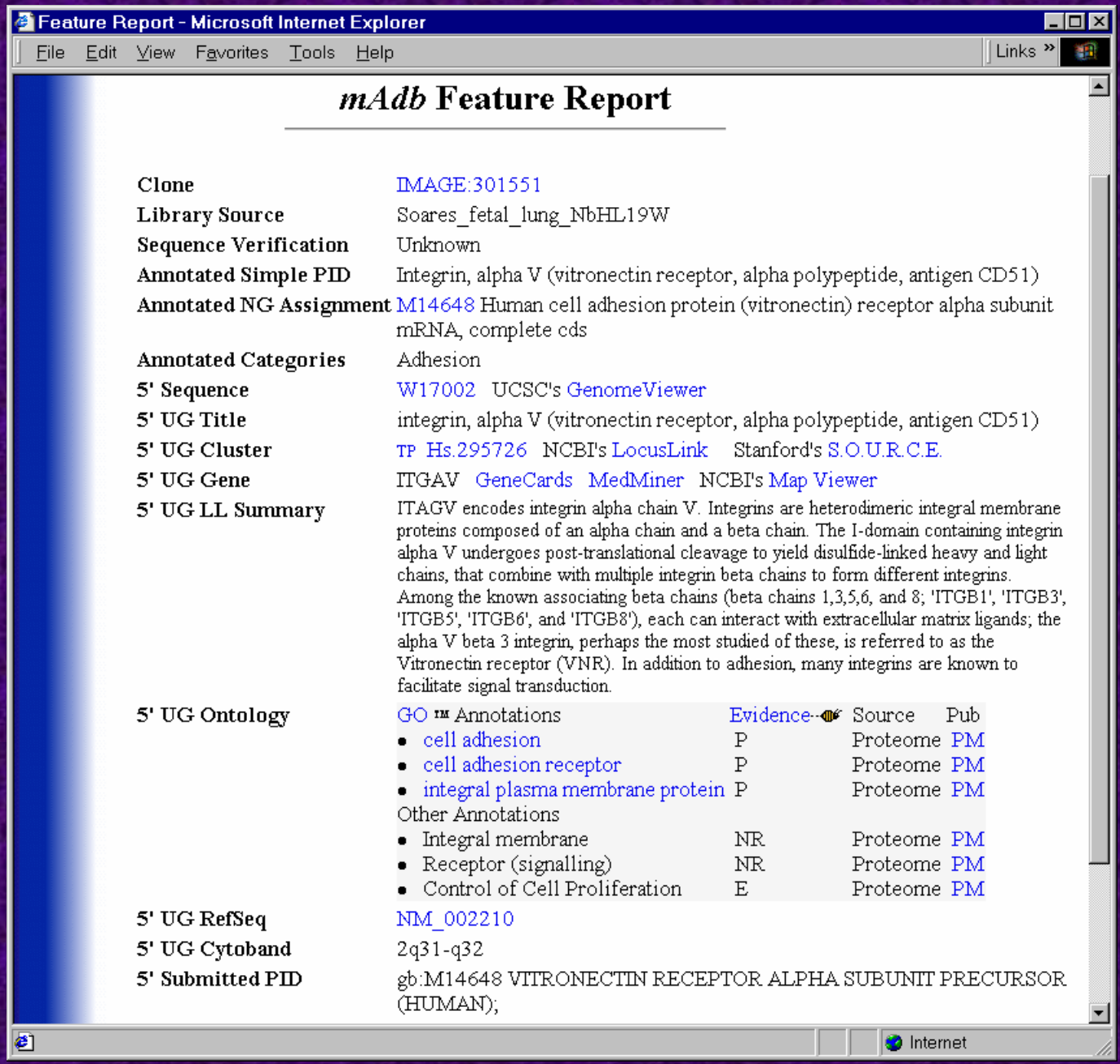
2. After the Dataset name (which can be **edit**ed with the link to the left), is the history of what was done in the preceding filtering step.

3. Go to the next page of the lab and scroll down to the bottom of the Web page.

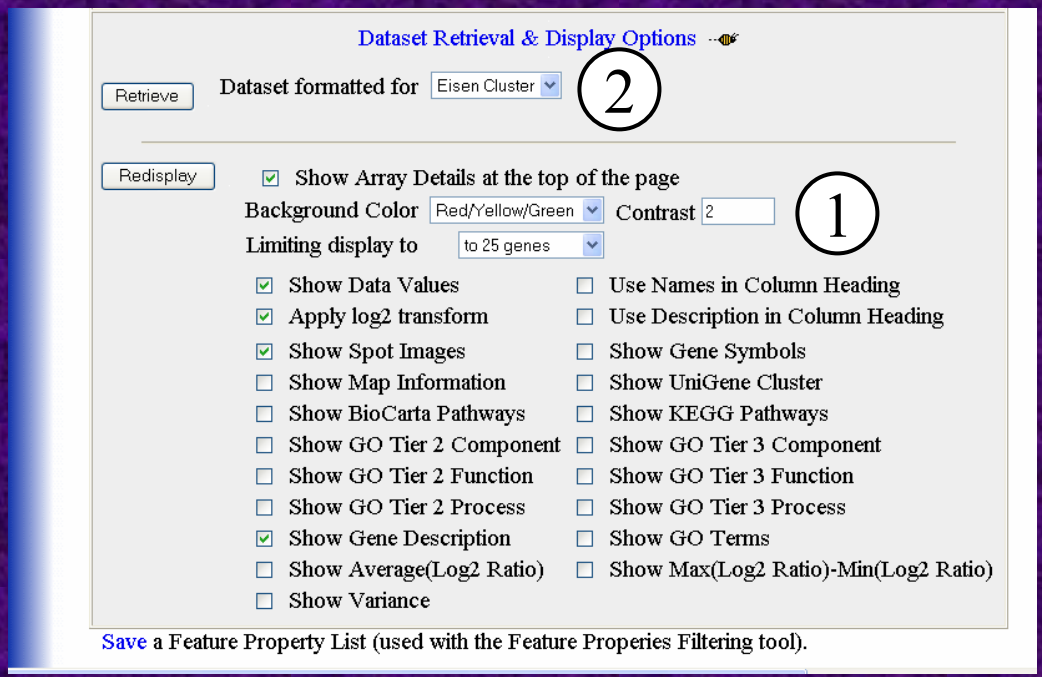
Lab 1. Main mAdb Dataset Display – Part 2



1. This is the main page to display expression data, and as we will see on the next page, is highly customizable. Each column represents an array, each row a gene feature. Grey boxes are either missing values or data that was filtered out due to low quality. You can page through the data using the **arrow** just above the columns of data.
2. The **Well ID** uniquely identifies the piece of DNA used on that feature, and the **Feature ID** is an accession number. The **Well ID** is a hyperlink to a montage of the spot images, whereas the **Feature ID** is a Hyperlink to a **Feature Report**, integrating information about the gene related to the feature and its function(s).
3. There is a brief description of the feature on the right hand side of the display. Note that each column can be sorted in either ascending or descending order using the **grey arrows** above each column.


Feature Report - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Links
mAdb Feature Report
Clone IMAGE:301551
Library Source Soares_fetal_lung_NbHL19W
Sequence Verification Unknown
Annotated Simple PID Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
Annotated NG Assignment M14648 Human cell adhesion protein (vitronectin) receptor alpha subunit mRNA, complete cds
Annotated Categories Adhesion
5' Sequence W17002 UCSC's GenomeViewer
5' UG Title integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
5' UG Cluster TP Hs.295726 NCBI's LocusLink Stanford's S.O.U.R.C.E.
5' UG Gene ITGAV GeneCards MedMiner NCBI's Map Viewer
5' UG LL Summary ITAGV encodes integrin alpha chain V. Integrins are heterodimeric integral membrane proteins composed of an alpha chain and a beta chain. The I-domain containing integrin alpha V undergoes post-translational cleavage to yield disulfide-linked heavy and light chains, that combine with multiple integrin beta chains to form different integrins. Among the known associating beta chains (beta chains 1,3,5,6, and 8; 'ITGB1', 'ITGB3', 'ITGB5', 'ITGB6', and 'ITGB8'), each can interact with extracellular matrix ligands; the alpha V beta 3 integrin, perhaps the most studied of these, is referred to as the Vitronectin receptor (VNR). In addition to adhesion, many integrins are known to facilitate signal transduction.
5' UG Ontology
GO ™ Annotations Evidence Source Pub
cell adhesion P Proteome PM
cell adhesion receptor P Proteome PM
integral plasma membrane protein P Proteome PM
Other Annotations
Integral membrane NR Proteome PM
Receptor (signalling) NR Proteome PM
Control of Cell Proliferation E Proteome PM
5' UG RefSeq NM_002210
5' UG Cytoband 2q31-q32
5' Submitted PID gb:M14648 VITRONECTIN RECEPTOR ALPHA SUBUNIT PRECURSOR (HUMAN);
Internet

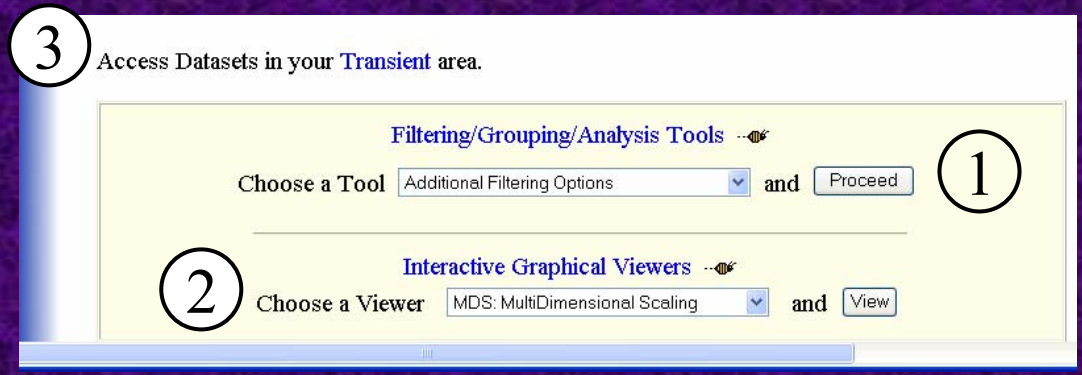
Lab 1. Main mAdb Dataset Display – Part 3



1. Here is where the data display on the preceding page can be customized, by checking or unchecking the checkboxes next to each column name. One can include numerical summary data (**Average(Log2 Ratio), Variance**, **Max(Log2 Ratio)-Min(log2 Ratio);** pathways (**KEGG, BioCarta**); Genome Ontology (**GO**) classifications; and display individual **Spot Images**, among others. One can also change or eliminate the **Background Color** on the table of data values, adjust its **Contrast** (the point where max red and green are reached), and also adjust how many genes are displayed in the table on a Web page (the default is 25). Once the choices are made, push the **Redisplay** button to refresh the page with your desired changes.

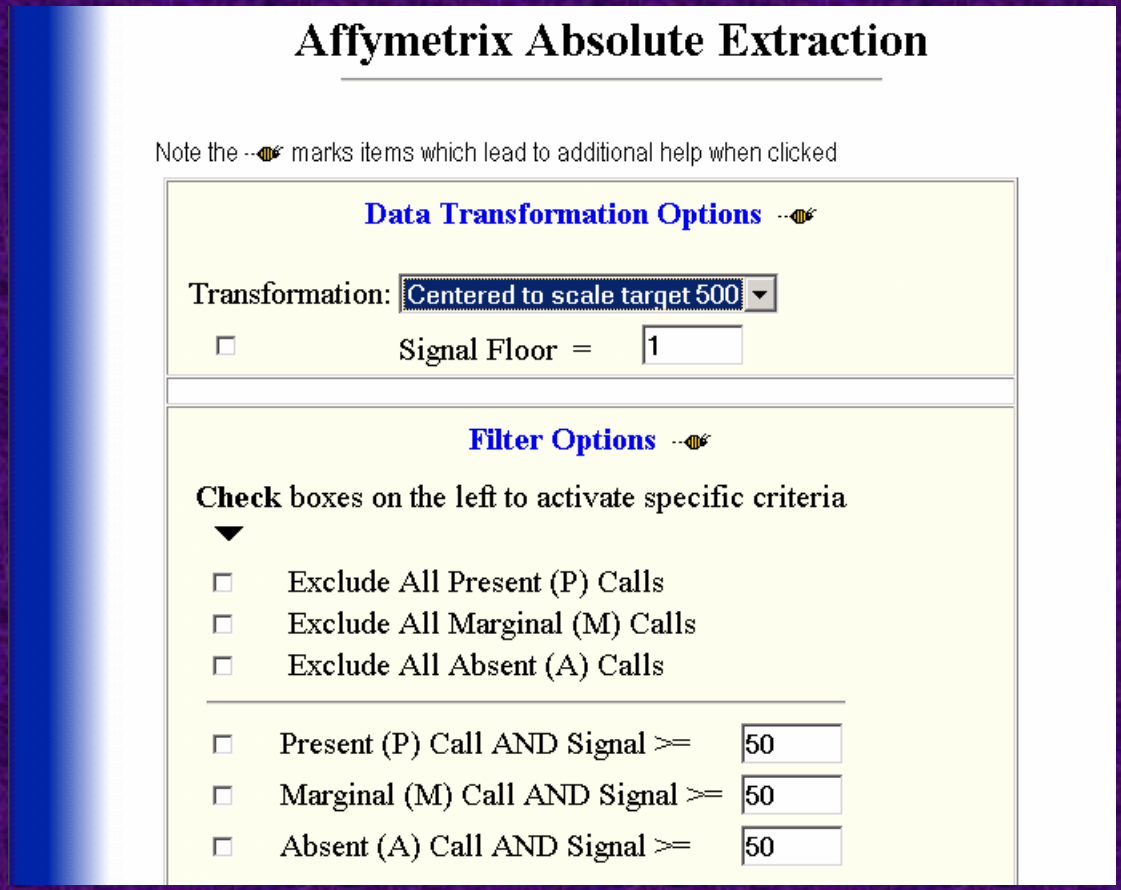
2. You can also retrieve the dataset for MS-Excel, the Eisen Cluster program format, or in tab-delimited files for the Macintosh, PC, or UNIX platforms.

Lab 1. Main mAdb Dataset Display – Part 4



1. Once the data is filtered by quality, the most likely next step is to do additional filtering and create a subset of this parent dataset. Under *Filtering/Grouping/Analysis Tools*, choose the default pulldown option of **Additional Filtering Options** and press **Proceed.**
2. Alternately, one could access *Interactive Graphical Viewers* from here, but we will return and demonstrate that later.
3. Also, you could **Access** other **Datasets in your Transient Area** from here with the link above the yellow panels.

Affy Extraction Tool (for Absolute data)



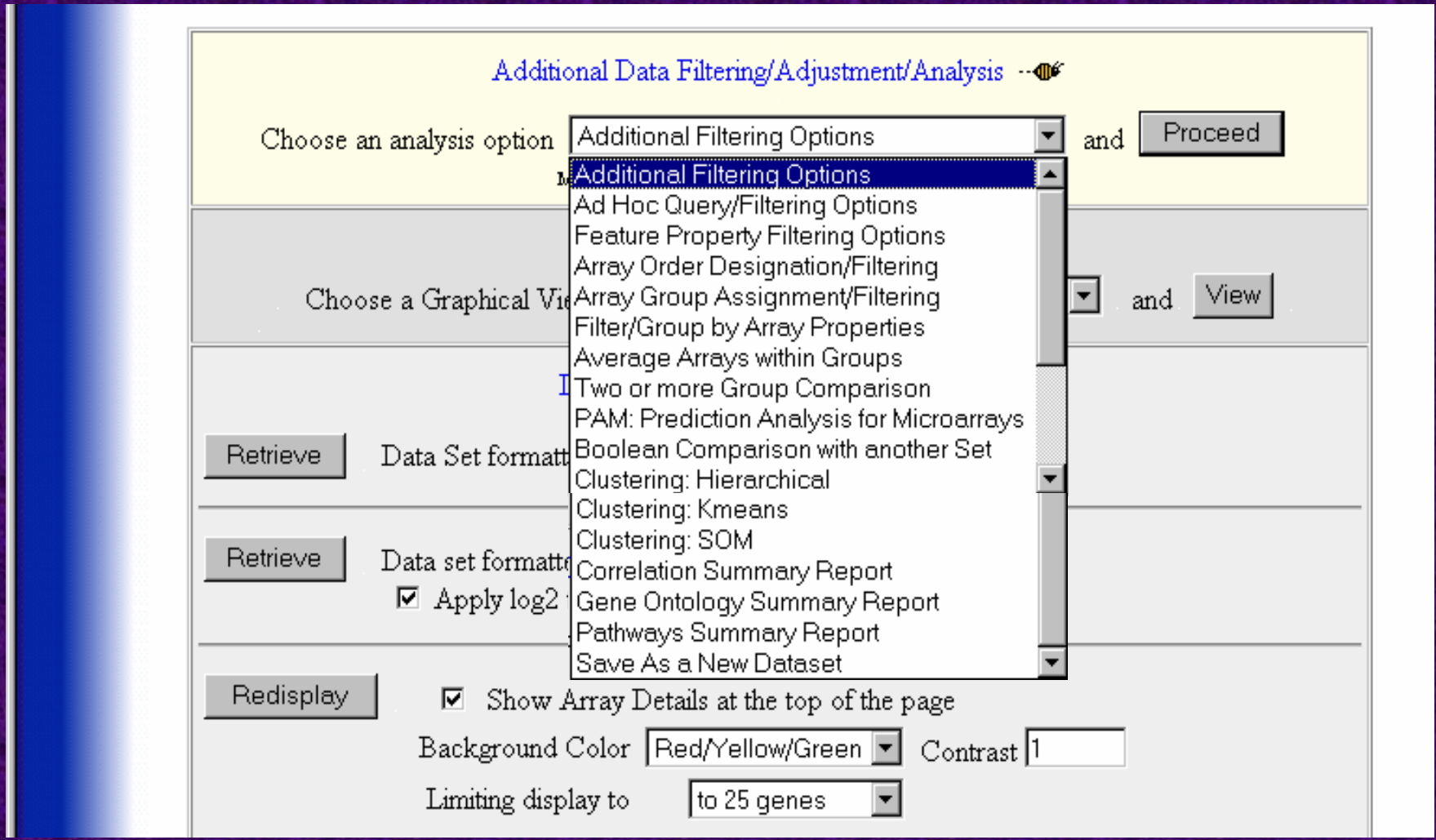
Break

V. Basic data analyses and dataset management

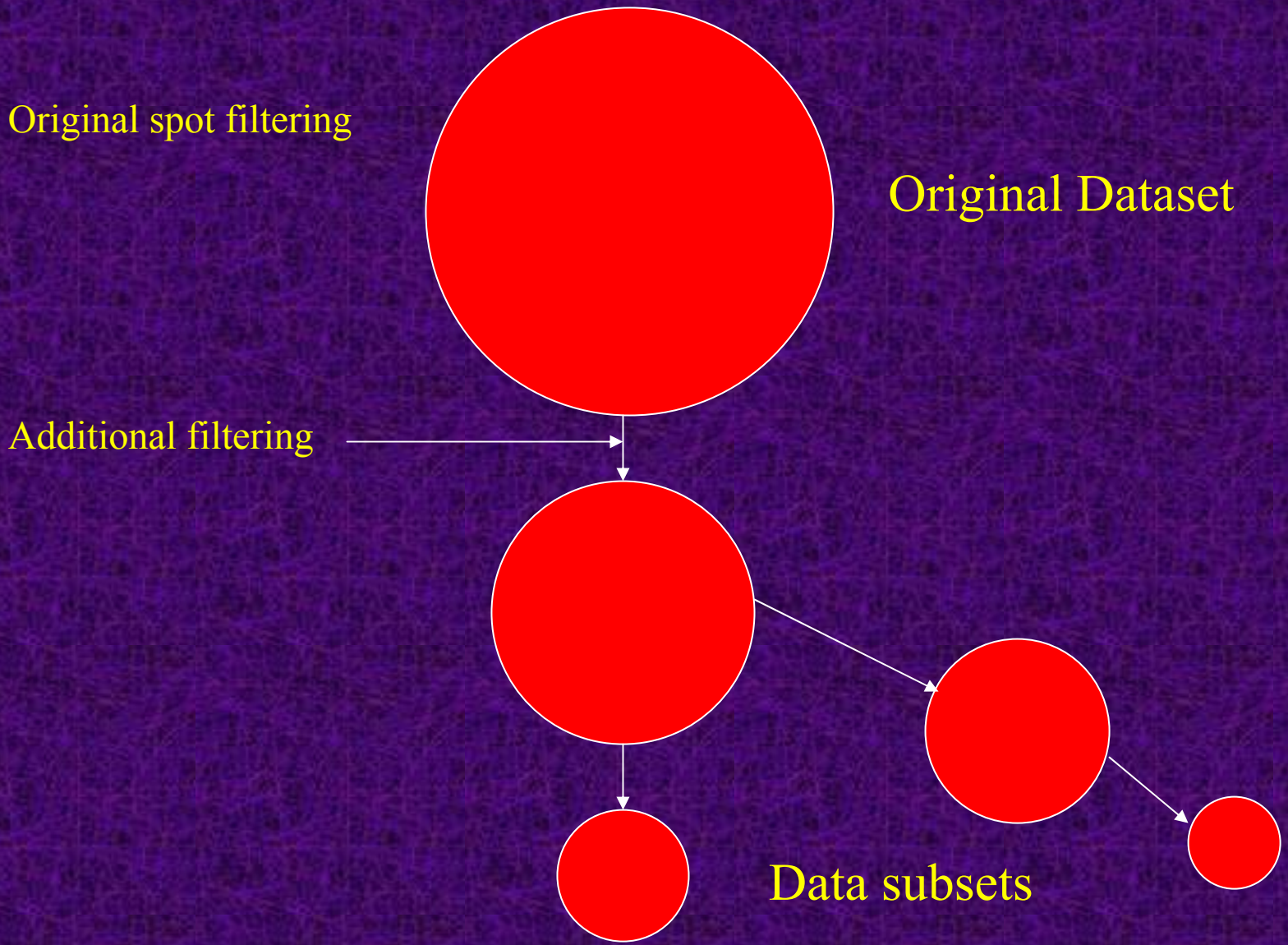
Tools for Basic Data Analyses

- Once you have a filtered dataset, you can:
  - Filter further for missing values and/or gene ratio levels
  - Do an *ad hoc* Keyword search
  - Filter datasets by lists of gene identifiers
  - View GO and Pathway Summaries
  - Use Graphic Tools
    - Interactive Scatter Plot
    - Correlation Summary Report
    - Multiple Array Viewer

Additional Filtering and Analysis Options



Dataset Structure -Filtering hierarchy /tree structure



Dataset History

12 Arrays and 3877 Expression Rows extracted with Spot Filter Options:
Chan A Spot size (percentage) >= 25 and Chan B Spot size (percentage) >= 25
AND ((Chan A Signal >= 50 AND Chan B Signal >= 50) OR (Chan B Signal >= 2500))
AND (Spots not flagged BAD or Not Found)
Note: For all GenePix results from Axon scanned arrays Chan A is CY3 and Chan B is CY5.
Rows ordered by Maximum(Ratio)/Minimum(Ratio) descending.

Thu Oct 4 09:22:48 EDT 2001

Interactive Array Filtering
12 arrays and 3877 genes in the original dataset
6 arrays and 3875 genes in the output data set.
6 Arrays were interactively excluded
2 Genes excluded for having zero observations in the resultant array set.

Thu Oct 4 09:23:56 EDT 2001

6 arrays, 3875 genes in the input dataset
941 Genes and 6 arrays passed filters
111 genes excluded for being present in less than 80% arrays.
2823 genes excluded where variance is in the lowest 75 percentile (Variance<0.44).

Link to the output Dataset

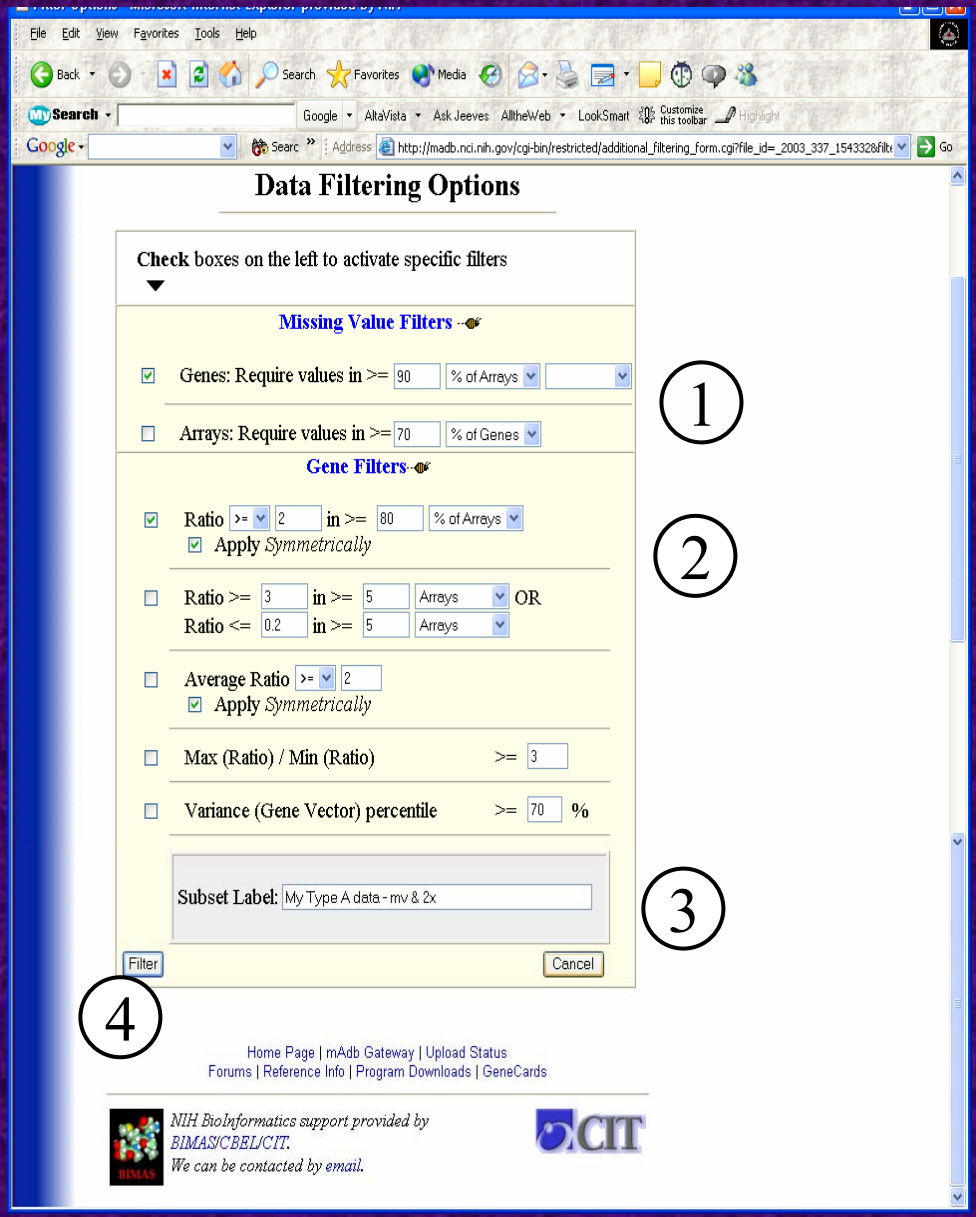
A log is maintained for each dataset tracing the analysis history. When the history is displayed, links are provided to allow the user to recall any dataset in the analysis chain.

Lab 2 –Additional Filtering

Goal Lab 2: To filter out missing values for genes and to look for genes up or down regulated at least 2-fold.

- Applies selected filtering options to the dataset based on values in the data and creates a new subset.
- For gene filters, ratios are expressed as fold changes and all calculations are done in log space

Lab 2. Additional Filtering



1. Filter the rows of data from the parent dataset for missing values, requiring genes in >= **80%** of Arrays. Alternately, it is possible to filter out Arrays by requiring values in >= 70% of genes, for example.
2. mAdb supports a wide variety of Gene Filters: We will use a **Ratio >= 2 in >= 80 % of Arrays**, with the **Apply *Symmetrically*** box checked to obtain genes up and down-regulated by 2-fold or more.

   Other options are:

   – Filter for at least 3 fold up in 5 or more arrays OR at least 5-fold down (0.2x up) in 5 or more arrays.

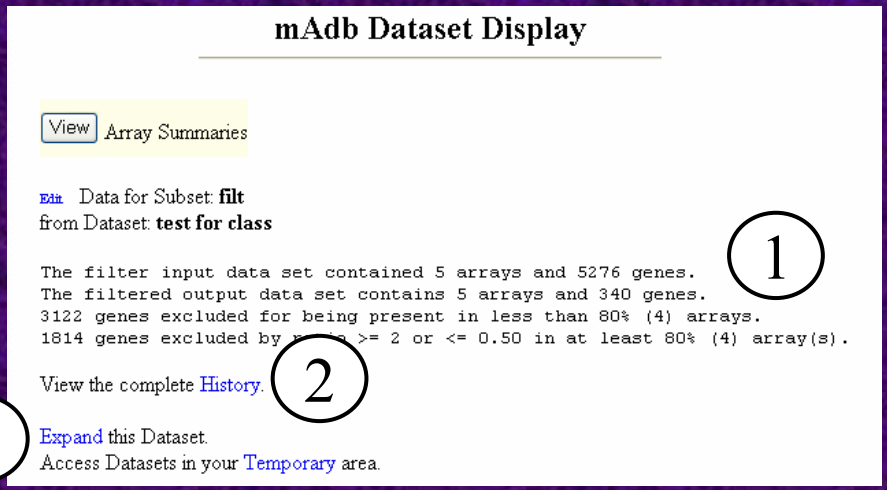
   – Filter for an average Ratio across the row at least two fold or more, applied symmetrically to obtain genes with an average ratio two-fold or more up or down regulated.

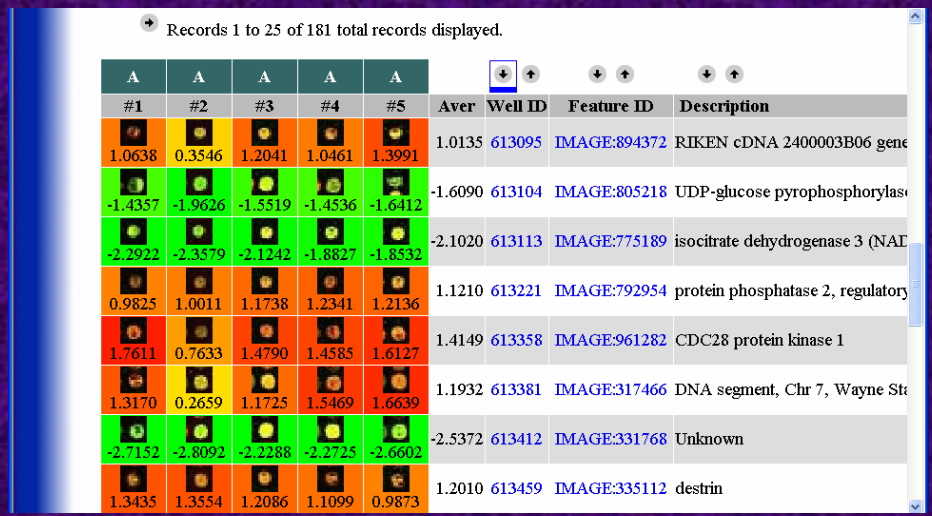
   – Filter for those rows showing a difference between the maximum ratio and minimum ratio on each row of 3 fold or more

   – Rank the genes by percentile of variance, and then filter for those genes in the top 30%ile of variance – ie. The genes that vary the most across the rows statistically.

   – N.B. Filters are applied in order from top to bottom on this page
3. Label the subset "**80% missing values & 2 fold up/down**" to reflect what you did .
4. Press the **Filter** button to continue and create the desired subset.

Lab 2. Additional Filtering





1. Note that in the returned dataset, there are many fewer missing values – see the history log for how many genes were filtered out to create this subset.
2. This is a data subset – you can view the complete History of the dataset via this link.
3. You can also **Expand this Dataset** to show the parent and all children, or again **Access Datasets in your Transient Area** via these links.

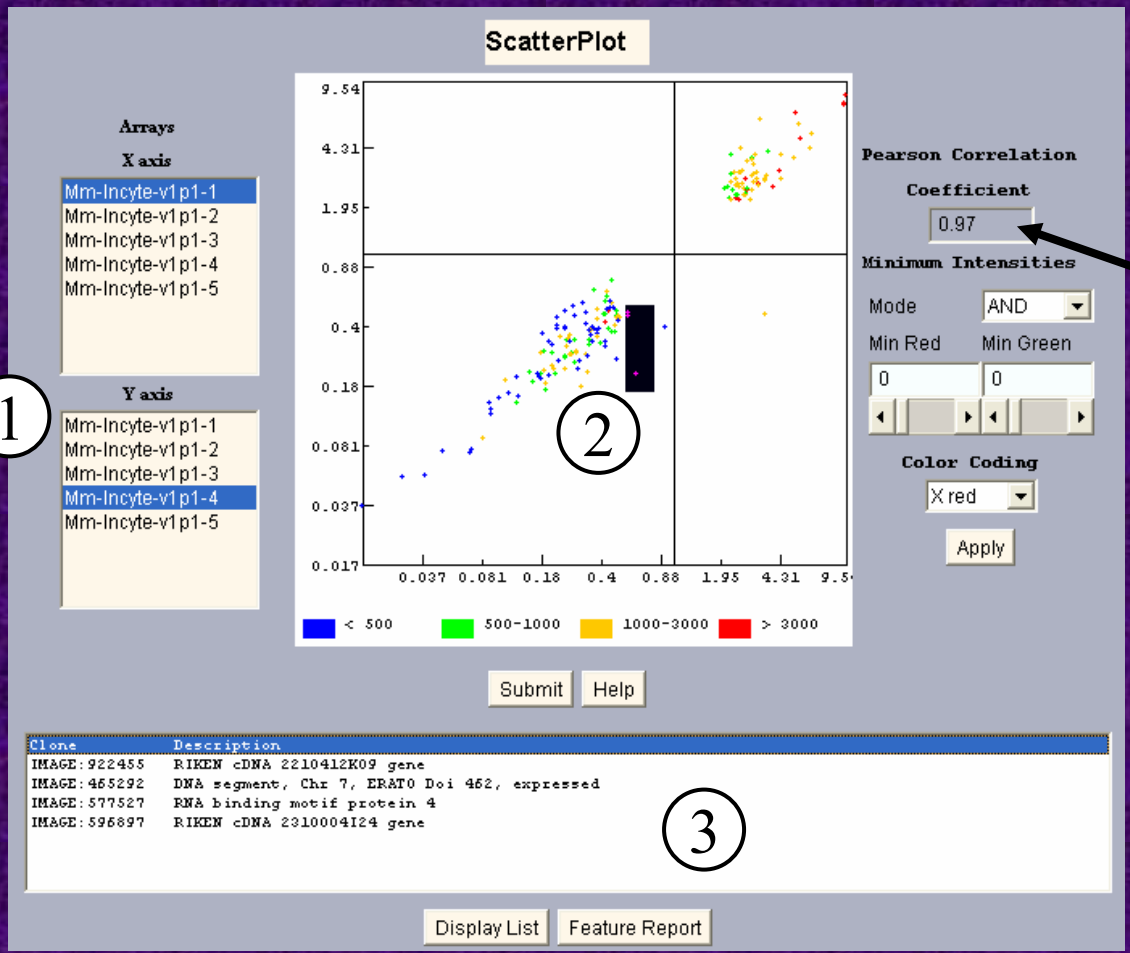
Notes:

- Applies selected filtering options to the dataset based on values in the data and creates a new subset.
- For gene filters, ratios are expressed as fold changes and all calculations are done in log space

Lab 3 – Using More Tools

Goals Lab 3: To use the Interactive Scatter Plot, Correlation Summary Report, Ad Hoc query, Multiple Array Viewer, Pathway, GO summary Reports and feature properties filtering,

Visualization Tools – Interactive Scatter Plot Applet



- Replicate experiments should be on a 45° angle (slope of 1) and the Pearson Correlation Coefficient should be approaching 1
- Reverse fluor experiments should have a Pearson Correlation Coefficient approaching -1

Access from *Interactive Graphical Viewers* Menu on main **mAdb Dataset Display** page:

1. Choose Arrays to be compared on X and Y axes
2. Can select outlying spots with mouse – genes will be shown in window below plot
3. Can get **Feature Report** by clicking on gene name in lower display box

Correlation Summary Report

Allows pair wise comparison of all arrays in a project – useful for comparing replicates and reverse fluors

mAdb Correlation Report

View Array Summaries

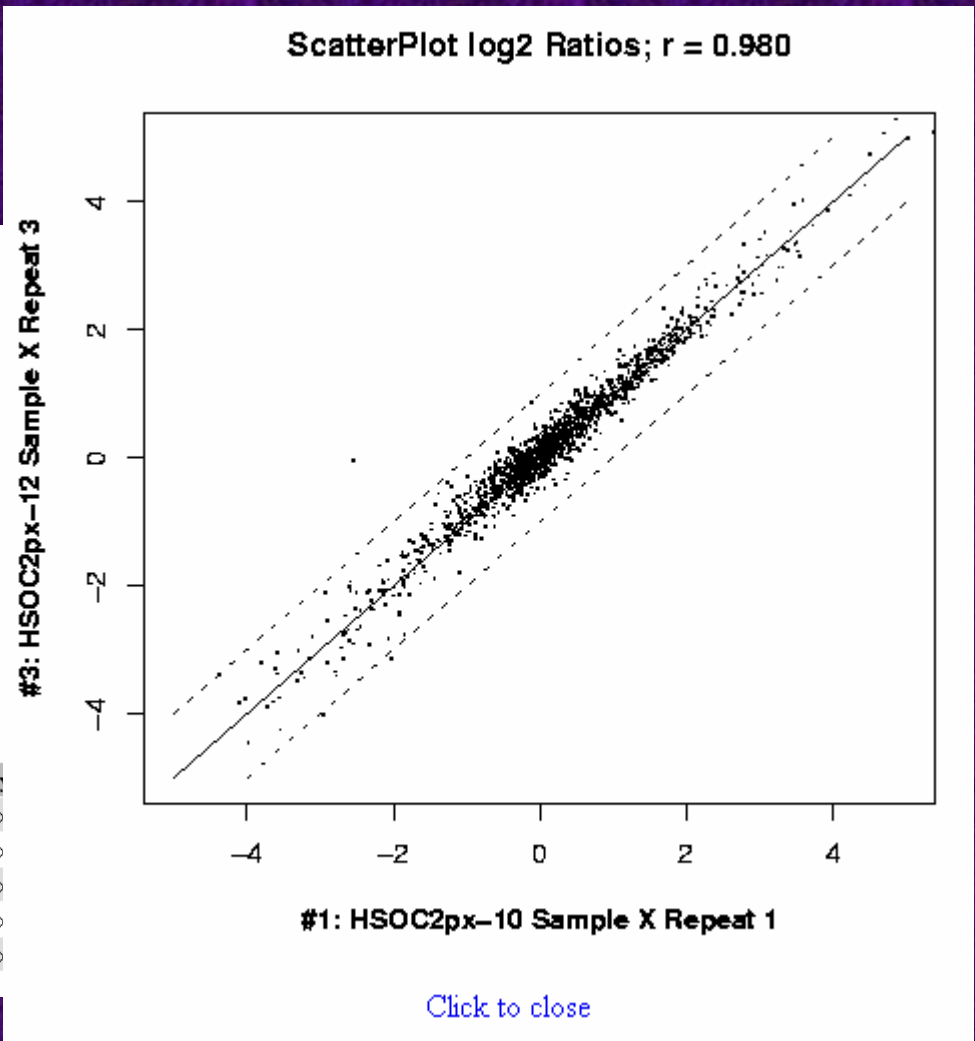
Return to the input dataset.

Redisplay | Background Color Scheme: - None - | Color Saturation Max/Mid/Min: 1 0 -1 | Note: For proper coloring Max > Mid > Min

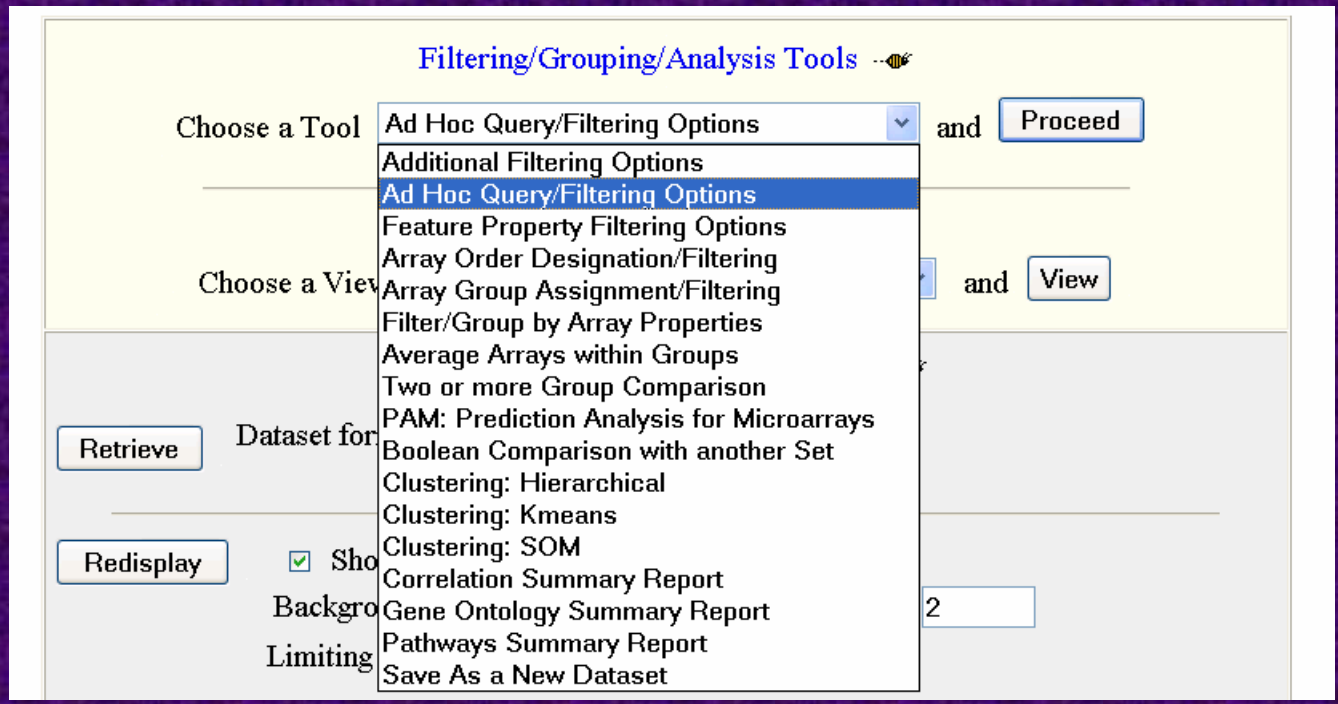
Note: Click on the Correlation values to display the corresponding ScatterPlot

Correlations

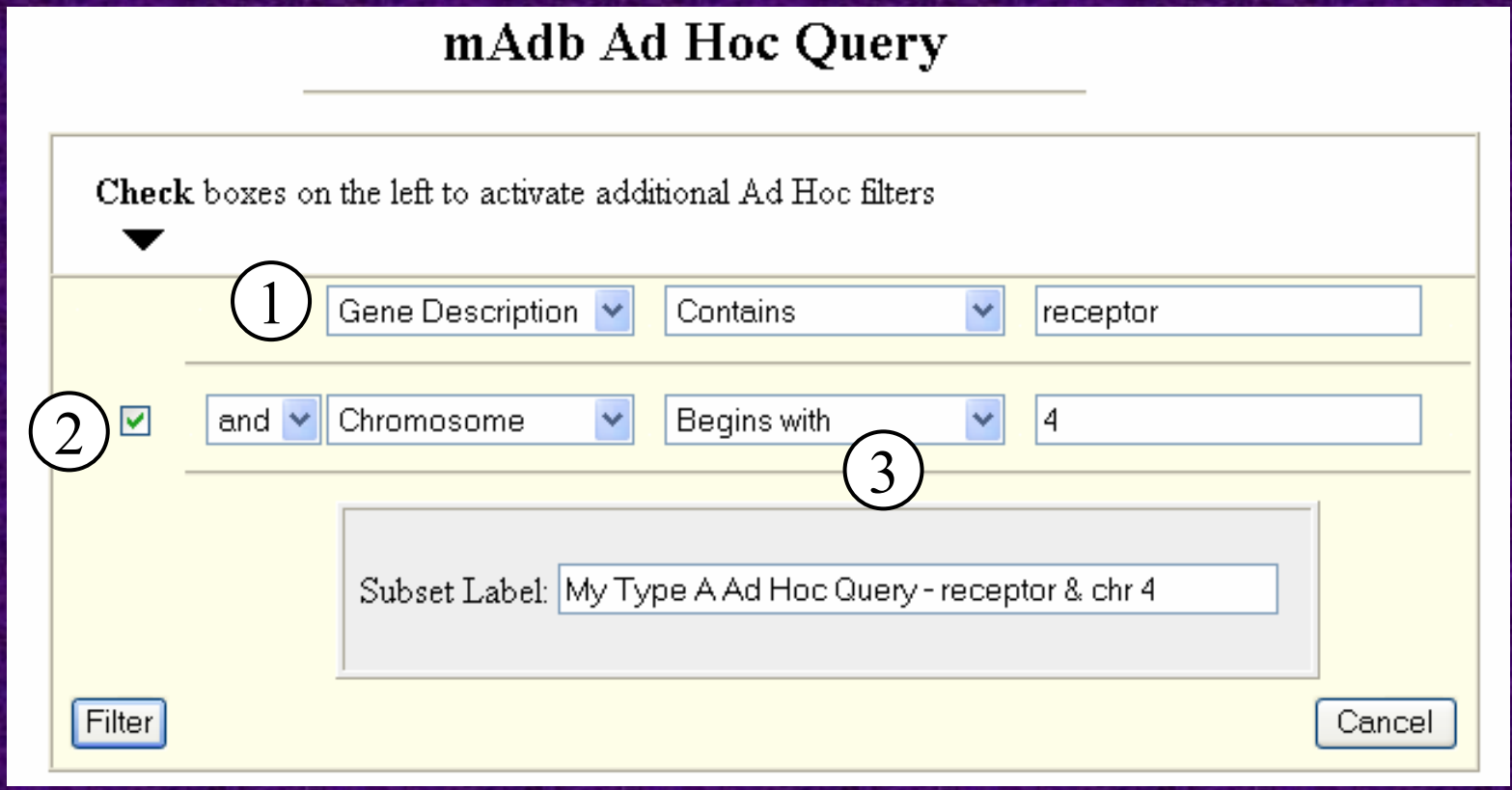
	A #1	A #2	A #3	A* #4	A* #5	Grp		Array Name	Array
1.	-	0.948	0.980	0.727	0.711	A	1.	HSOC2px-10	Samp
2.		-	0.928	0.742	0.738	A	2.	HSOC2px-11	Samp
3.			-	0.764	0.756	A	3.	HSOC2px-12	Samp
4.				-	0.972	A*	4.	HSOC2px-14	Samp
5.					-	A*	5.	HSOC2px-15	Samp



Selecting Ad Hoc Query Tool



Select “Ac



Basically a Boolean Keyword search; access from main **mAdb Dataset Display** page tool pulldown menu:

1. Can pick from **BioCarta Pathway, Feature ID, Gene Description, Gene Symbol, GO term, KEGG Pathway, Map Location, UniGene ID, Well ID**
2. Check box to add another term with **AND/OR** choice
3. Can choose **Contains, Begins With, Equals, Does Not Contain, Does Not Begin With, Does Not Equal** for search qualifier

Output of Ad Hoc Query

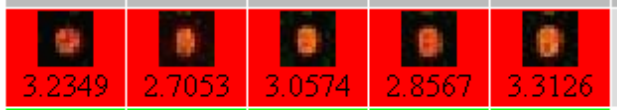
mAdb Dataset Display

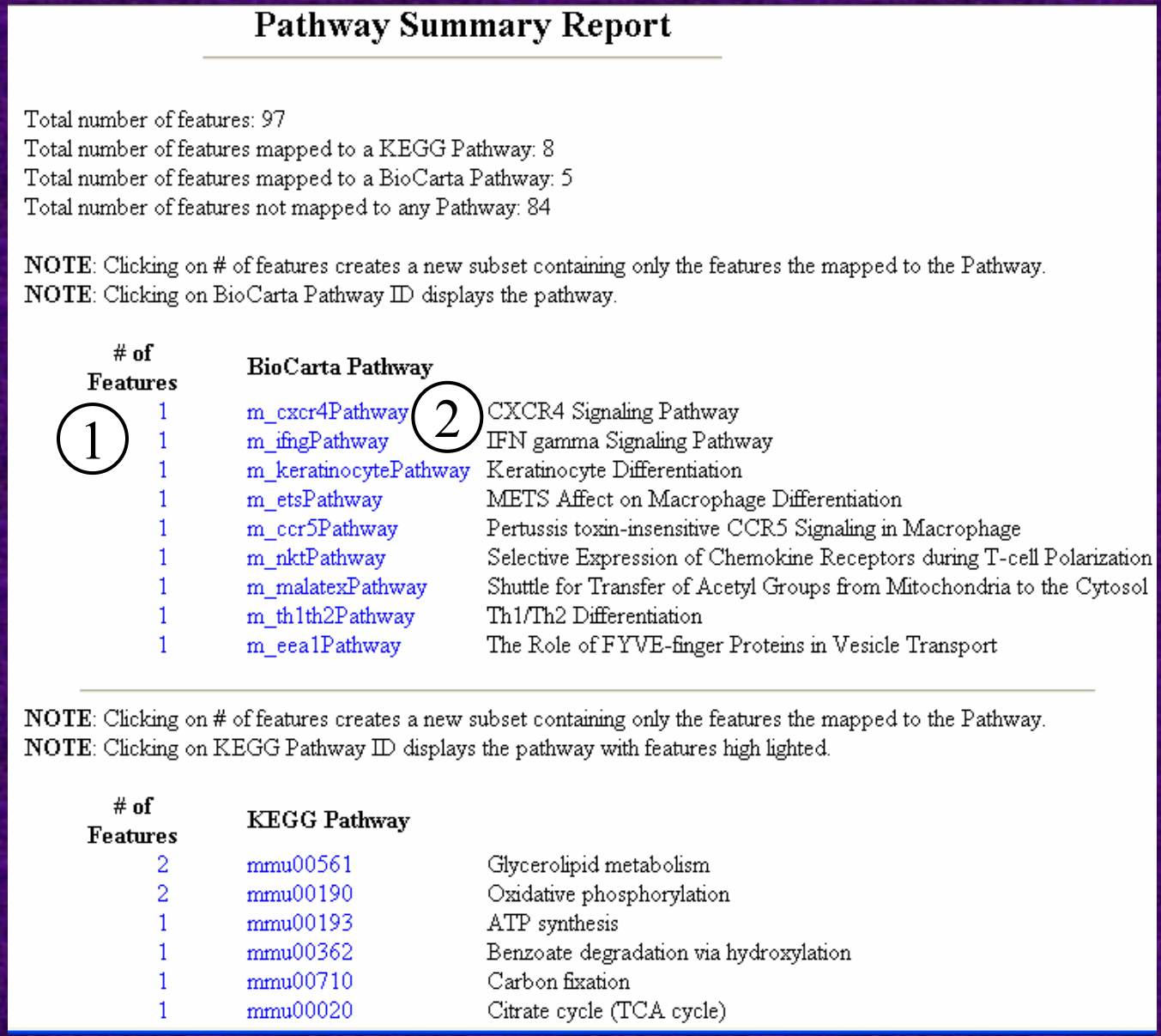
View Array Summaries

Edit Data for Subset: **My Type A Ad Hoc Query - receptor & chr 4**
from Dataset: **test for class**

```
Ad Hoc Filtering
5 arrays and 340 genes in the input dataset
5 arrays and 2 genes in the output dataset.
Ad Hoc Filter:
    Gene Description Contains 'receptor'
AND Chromosome Begins with '4'
```

Records 1 to 2 of 2 total records displayed.

A	A	A	A	A					
#1	#2	#3	#4	#5	Aver	Well ID	Feature ID	Map	Description
 3.2349	2.7053	3.0574	2.8567	3.3126	3.0334	614354	IMAGE:403453	4 C6-D1	protein tyrosine phosphatase, receptor type, F
 -1.9201	-2.4286	-1.7173	-1.9279	-1.8618	-1.9711	620446	IMAGE:735186	4 D2.3	nuclear receptor binding factor 1



Pathway Summary Report

Total number of features: 97
Total number of features mapped to a KEGG Pathway: 8
Total number of features mapped to a BioCarta Pathway: 5
Total number of features not mapped to any Pathway: 84

NOTE: Clicking on # of features creates a new subset containing only the features the mapped to the Pathway.
NOTE: Clicking on BioCarta Pathway ID displays the pathway.

# of Features	BioCarta Pathway	
1	m_cxcr4Pathway	CXCR4 Signaling Pathway
1	m_ifngPathway	IFN gamma Signaling Pathway
1	m_keratinocytePathway	Keratinocyte Differentiation
1	m_etsPathway	METS Affect on Macrophage Differentiation
1	m_ccr5Pathway	Pertussis toxin-insensitive CCR5 Signaling in Macrophage
1	m_nktPathway	Selective Expression of Chemokine Receptors during T-cell Polarization
1	m_malatexPathway	Shuttle for Transfer of Acetyl Groups from Mitochondria to the Cytosol
1	m_th1th2Pathway	Th1/Th2 Differentiation
1	m_eea1Pathway	The Role of FYVE-finger Proteins in Vesicle Transport

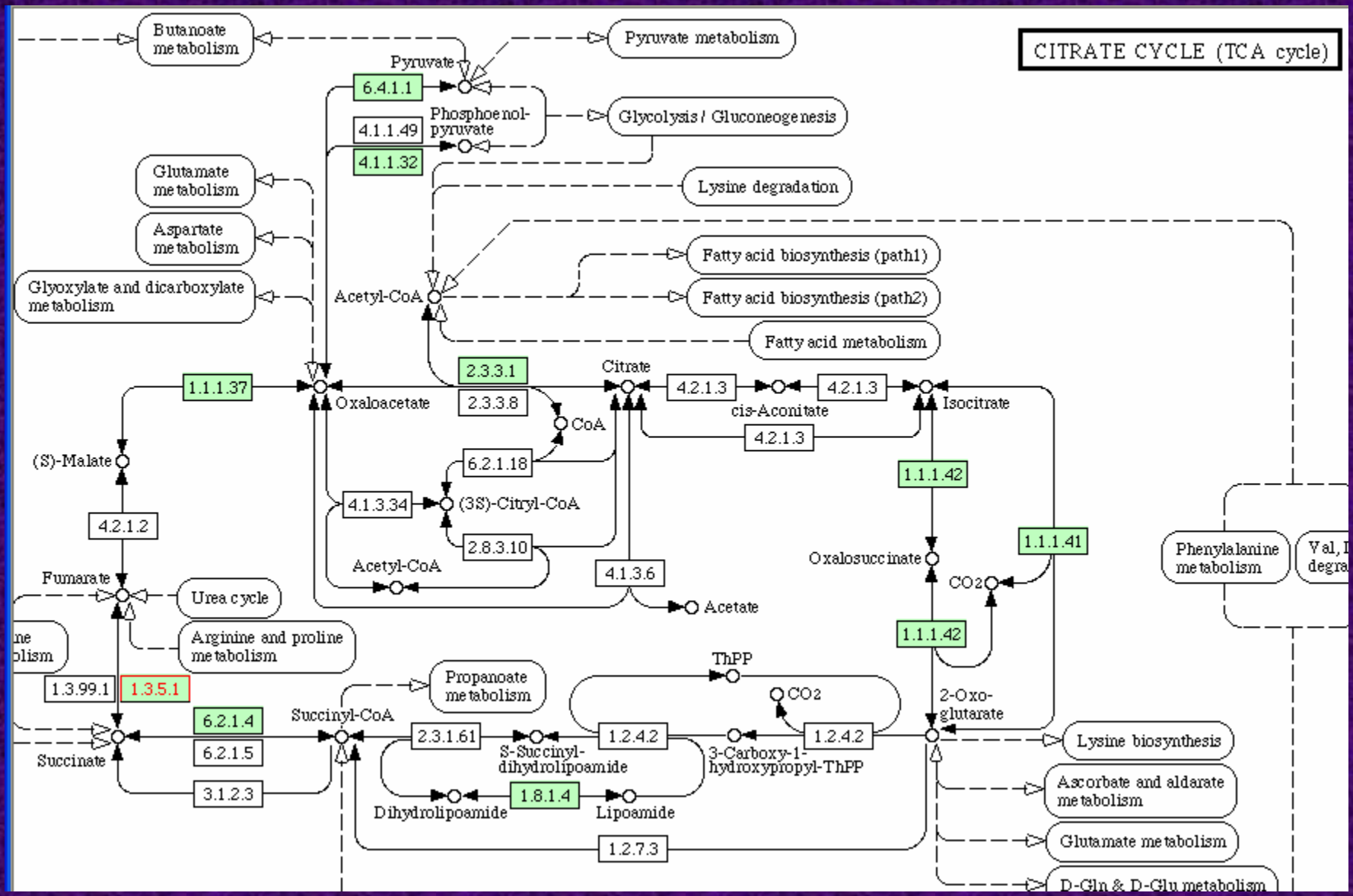
NOTE: Clicking on # of features creates a new subset containing only the features the mapped to the Pathway.
NOTE: Clicking on KEGG Pathway ID displays the pathway with features high lighted.

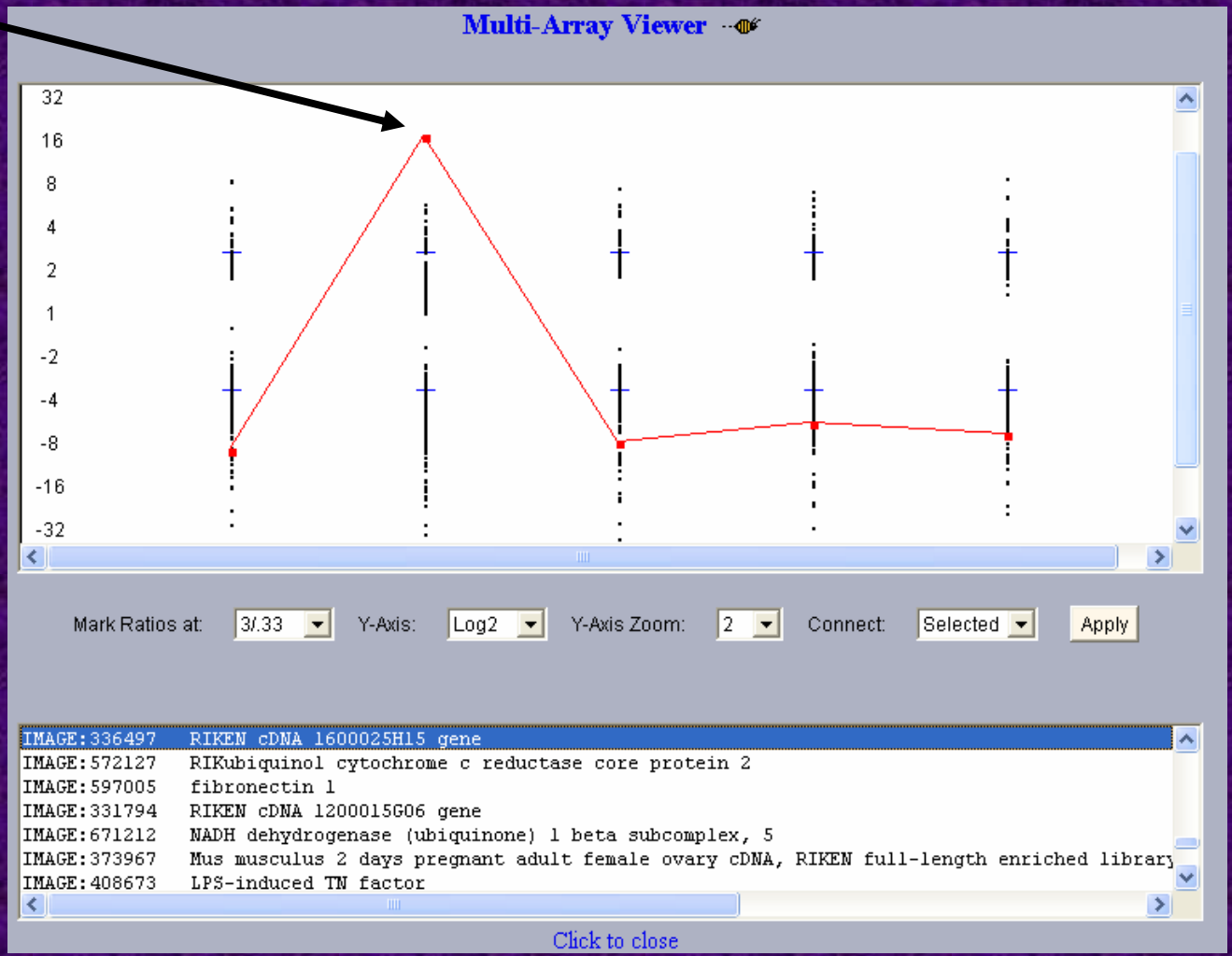
# of Features	KEGG Pathway	
2	mmu00561	Glycerolipid metabolism
2	mmu00190	Oxidative phosphorylation
1	mmu00193	ATP synthesis
1	mmu00362	Benzoate degradation via hydroxylation
1	mmu00710	Carbon fixation
1	mmu00020	Citrate cycle (TCA cycle)

Access from main tools menu on **mAdb Dataset Display** Page:

1. Clicking on **# of Features** link creates a new dataset of just those features.
2. Clicking on **BioCarta Pathway** links show pathway on BioCarta Web site.
3. Also have GO Ontology Summary Report.

A KEGG Pathway

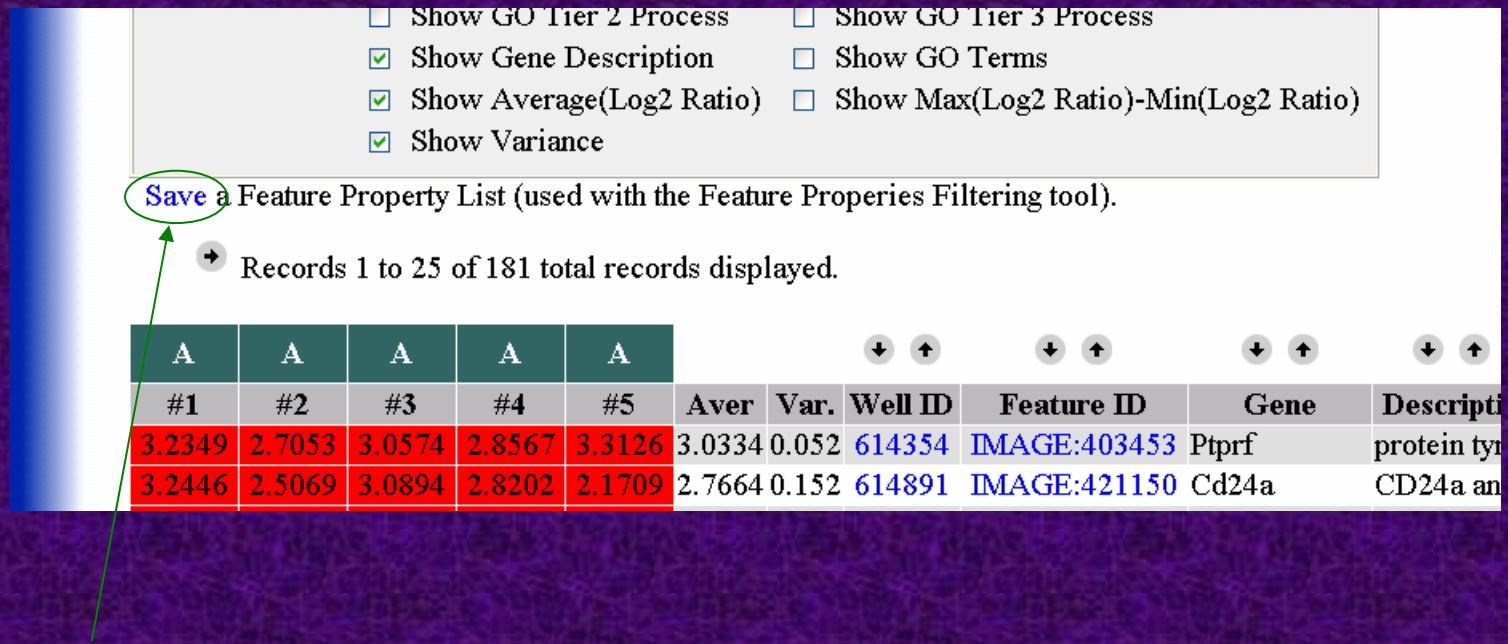




Access from *Interactive Graphical Viewers* Menu on main **mAdb Dataset Display** page :

1. Can choose a point on graphical window to display a graph of that gene's expression which passes through that point
2. Can select a gene name on lower list and graph will appear in plot above
3. Can get **Feature Report** by clicking on gene name in lower display box

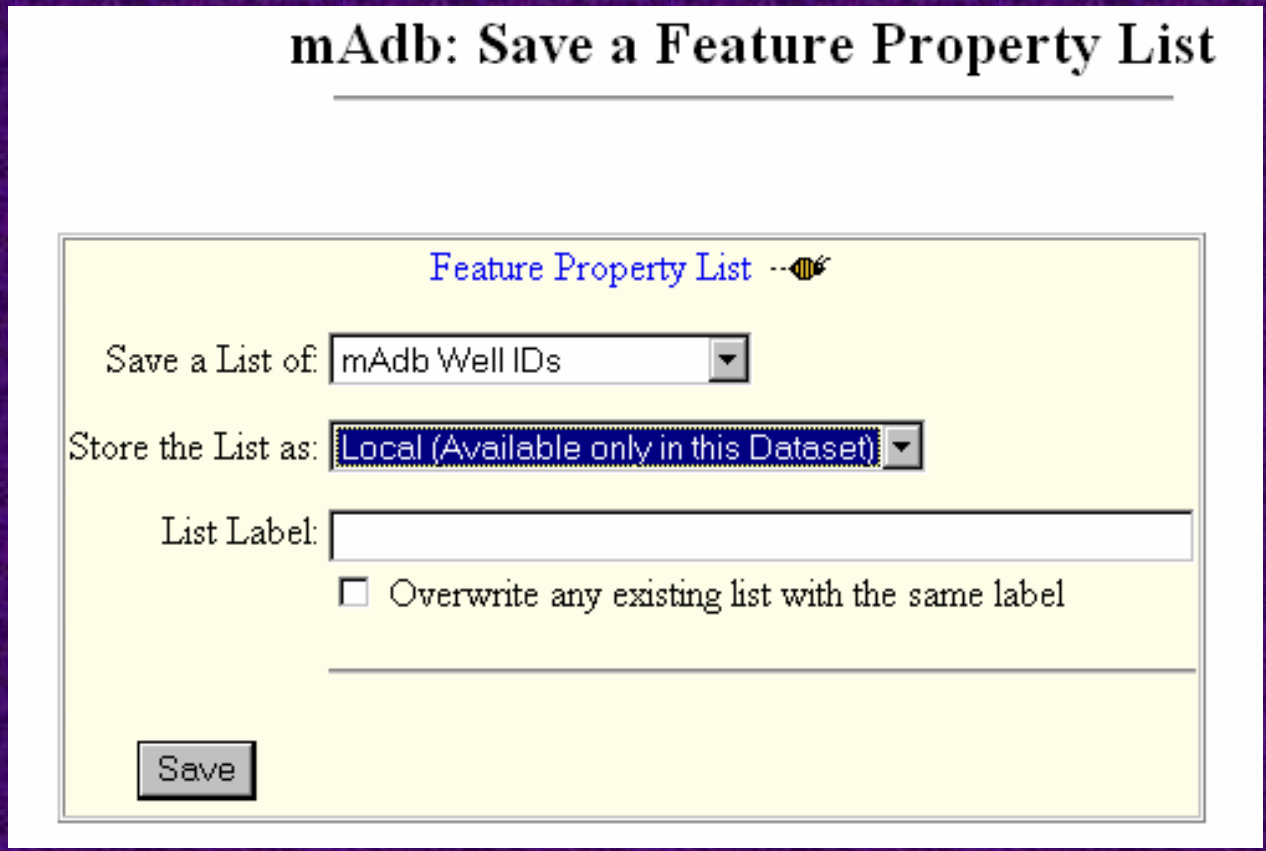
Save Feature Property List from mAdb Data Display



- Can save a list of well IDs, clone/feature identifiers, gene symbols, UniGene identifiers from the dataset display page

- List can be stored as local to the dataset or globally available to all datasets

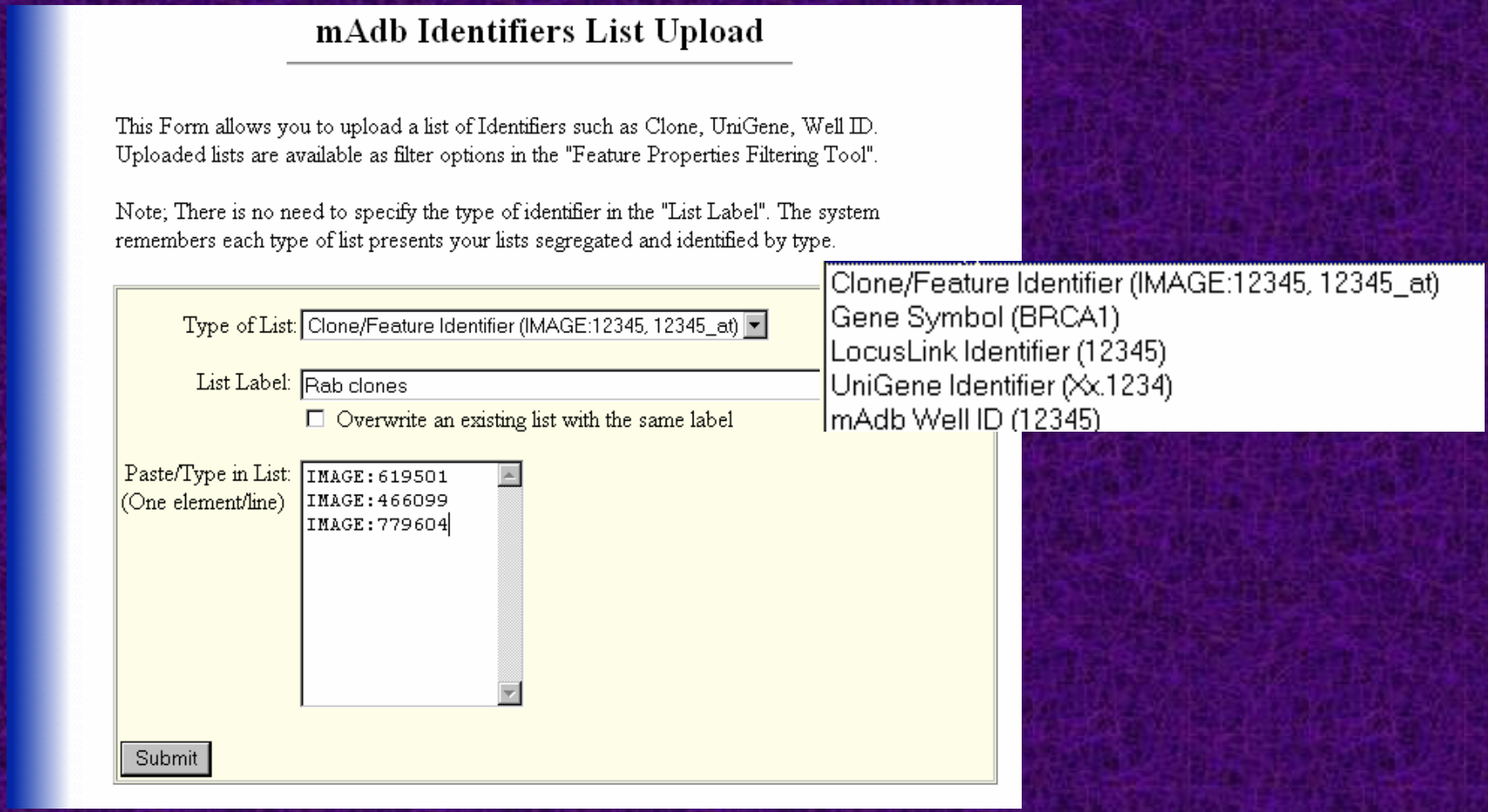
Save Feature Property List from mAdb Data Display



- Can save a list of well IDs, clone/feature identifiers, gene symbols, UniGene identifiers from the dataset

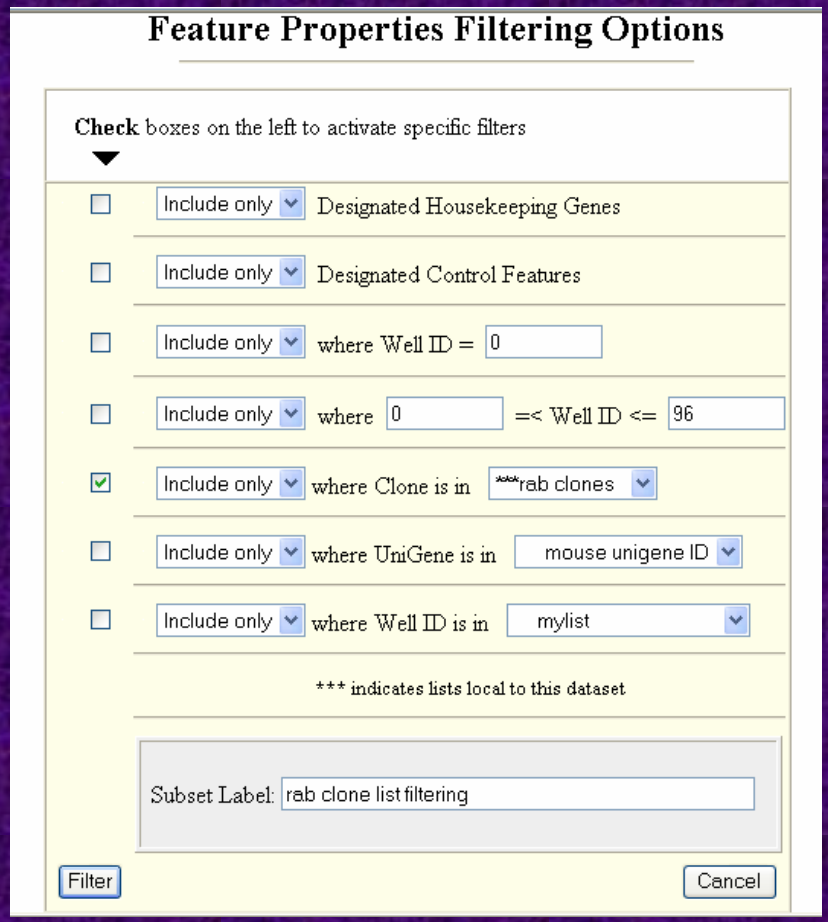
- List can be stored as local to the dataset or globally available to all datasets

Or Manually Create a List of Identifiers for Filtering



Can paste in list of identifiers; must use format as shown in pull down menu

Additional Filtering by Feature Properties and/or Lists

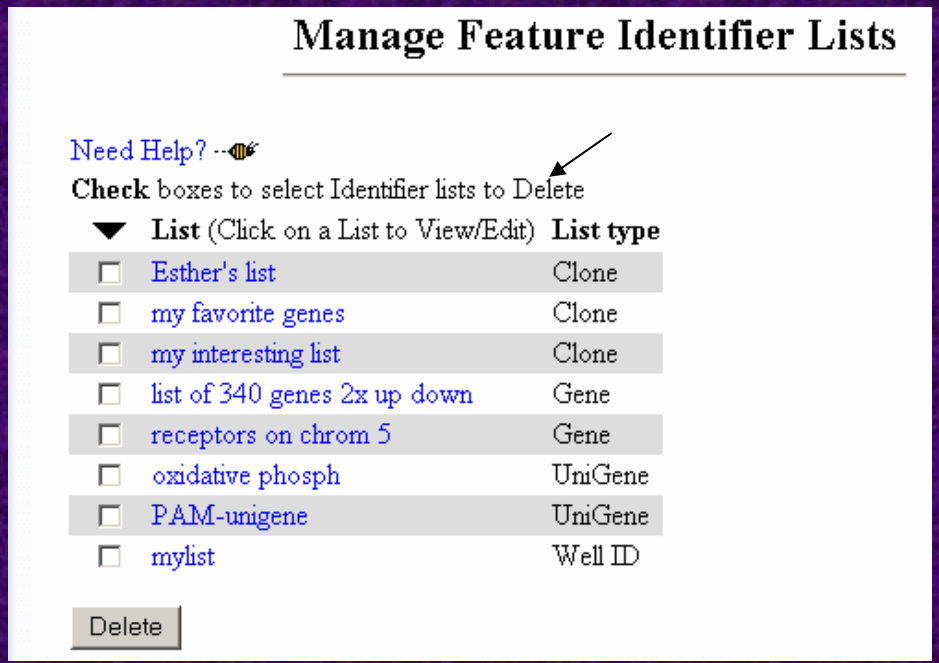


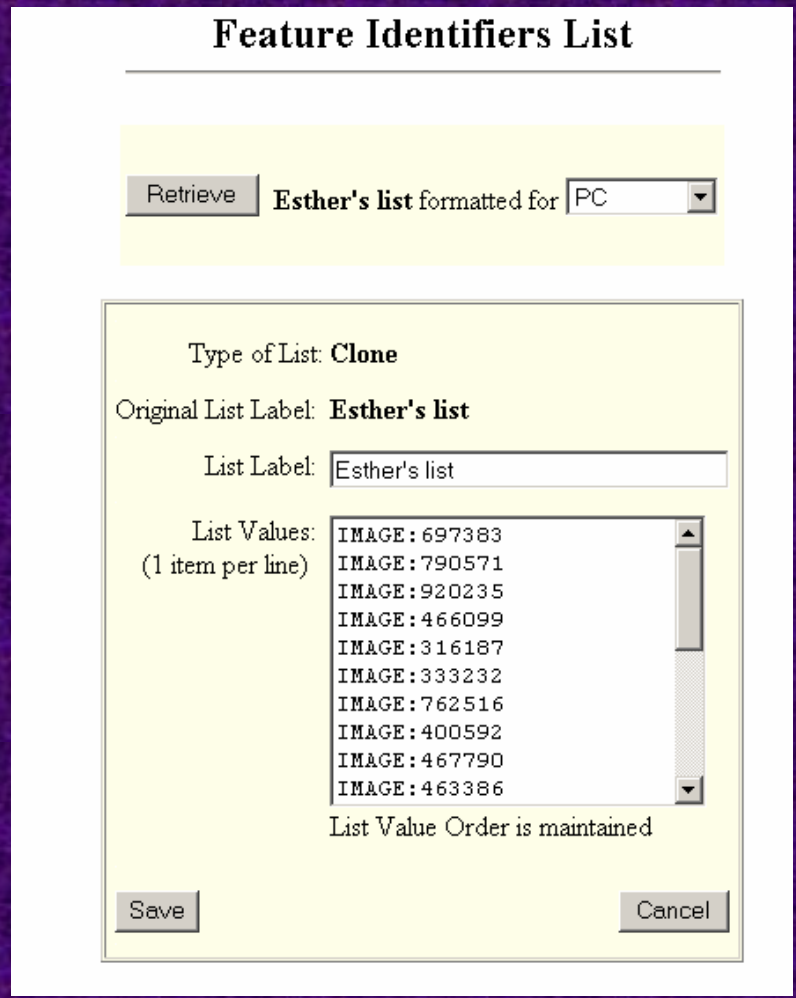
Filters another dataset so that only those clones matching feature properties or in selected lists are returned

Records 1 to 3 of 3 total records displayed.

A	A	A	A	A	B	B	B	B	B				
#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	Aver	Well ID	Feature ID	Description
1.4976	1.1814	1.6701	1.4505	1.4512	0.3770	0.4494	1.0830	1.1677	1.1442	1.1472	615892	IMAGE:466099	RAB6, member RAS oncogene family
	0.5956	0.1952	0.5601	0.2995	-0.2228	-0.0173	0.1918	0.0718	0.3550	0.2254	618176	IMAGE:619501	RAB1, member RAS oncogene family
0.2911	0.4528	0.3646	0.3658	-0.1094	-0.2409	-0.0061	-0.1560	0.2725	-0.0365	0.1198	613367	IMAGE:779604	RAB7, member RAS oncogene family

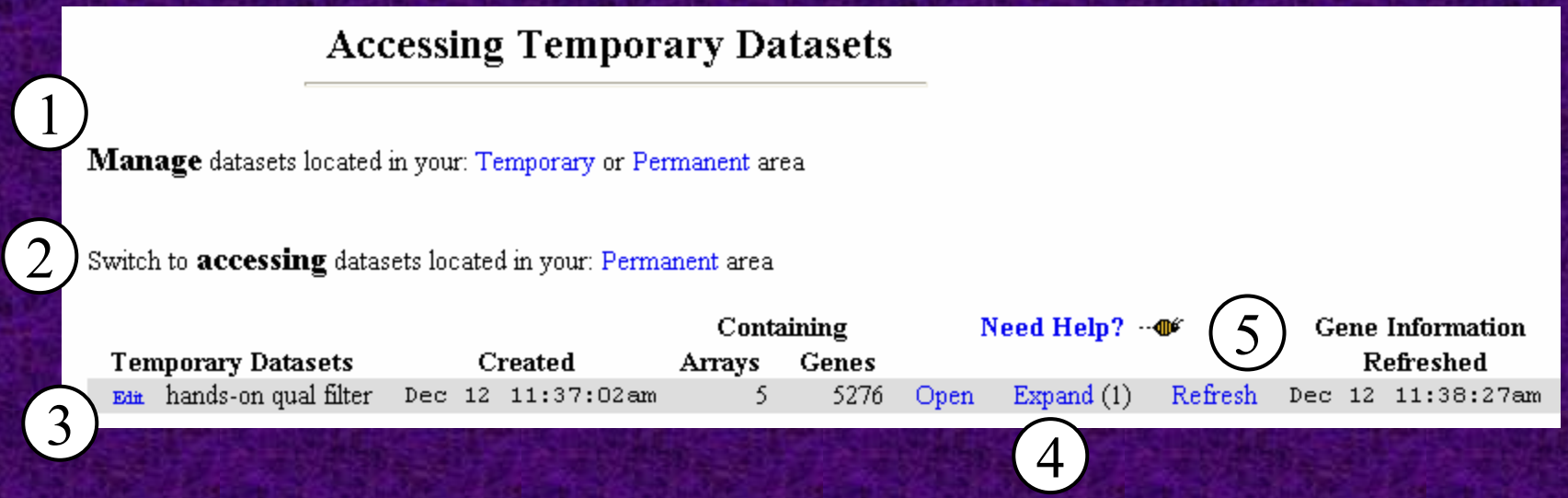
Managing Feature Lists





Lab 4 – Dataset Management

Goal: To manage datasets by renaming, moving its storage location or deleting them.



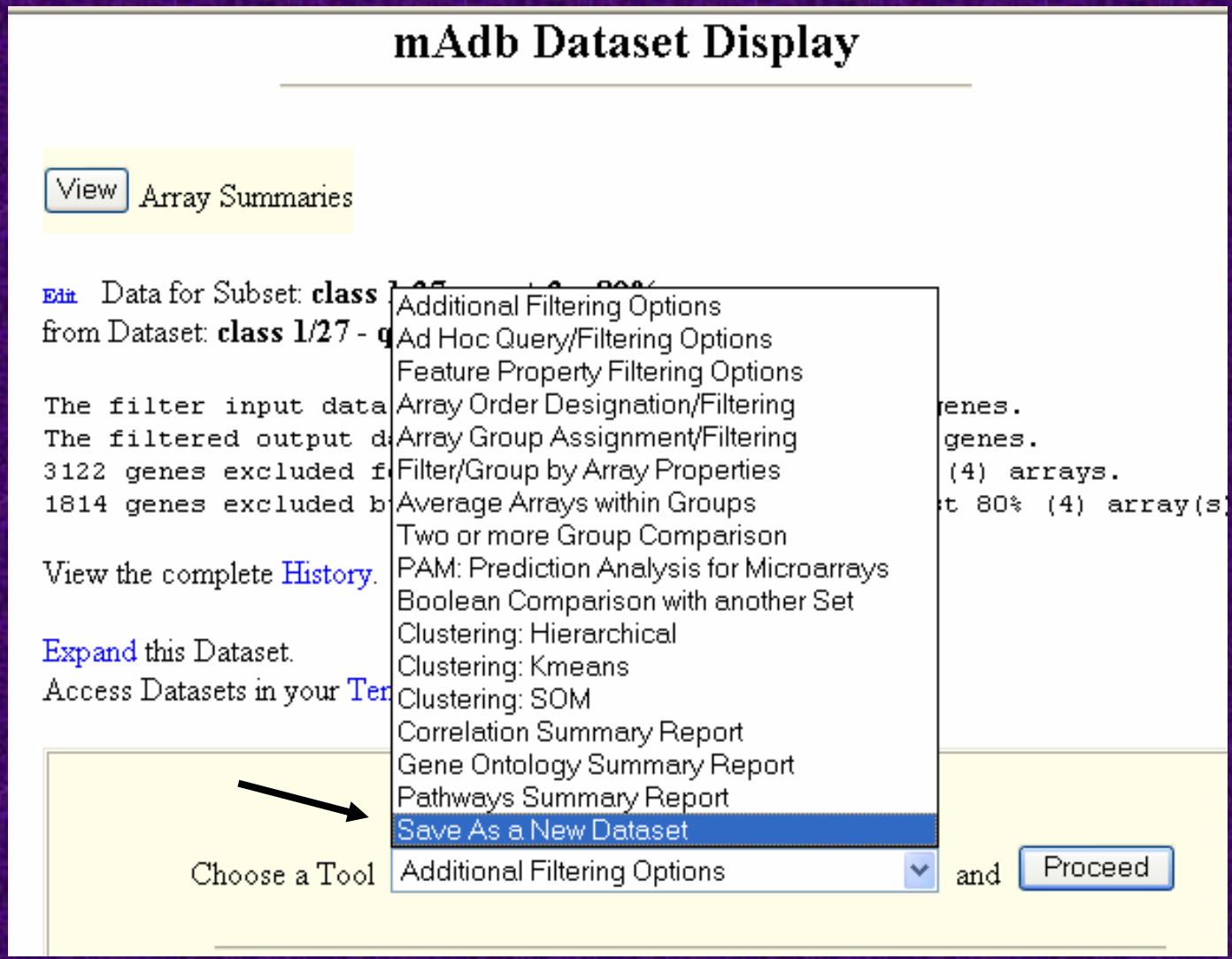
Dataset Access:

1. Can **Manage <u>Transient</u>**, **<u>Temporary</u>**, or **<u>Permanent</u>** Areas (wait for next slide)
2. Can **Access** other dataset areas which contain data (i.e. **<u>Permanent</u>**)
3. Can **Edit** dataset name
4. Can **Expand** to see parent dataset and all children of that parent
5. Can **Refresh** Gene Information – see next slide

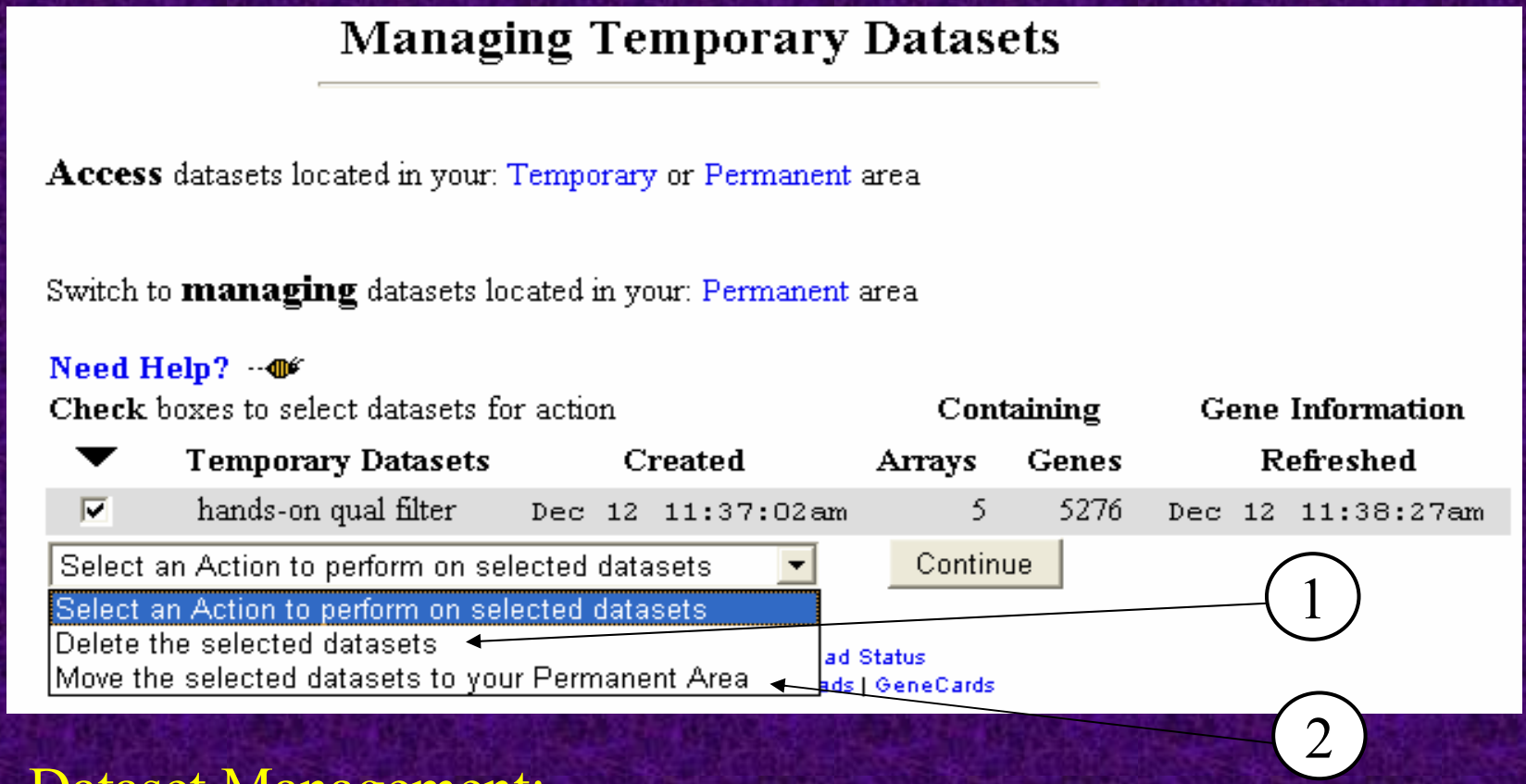
Refreshing Gene Information

- Clicking “refresh” link updates all of the gene information in the dataset (UniGene cluster, Description, Pathway info, Map info…)
- May want to “Save as a New Dataset”, and then refresh, if you don’t want gene names to change as you near publication

Save as New Dataset

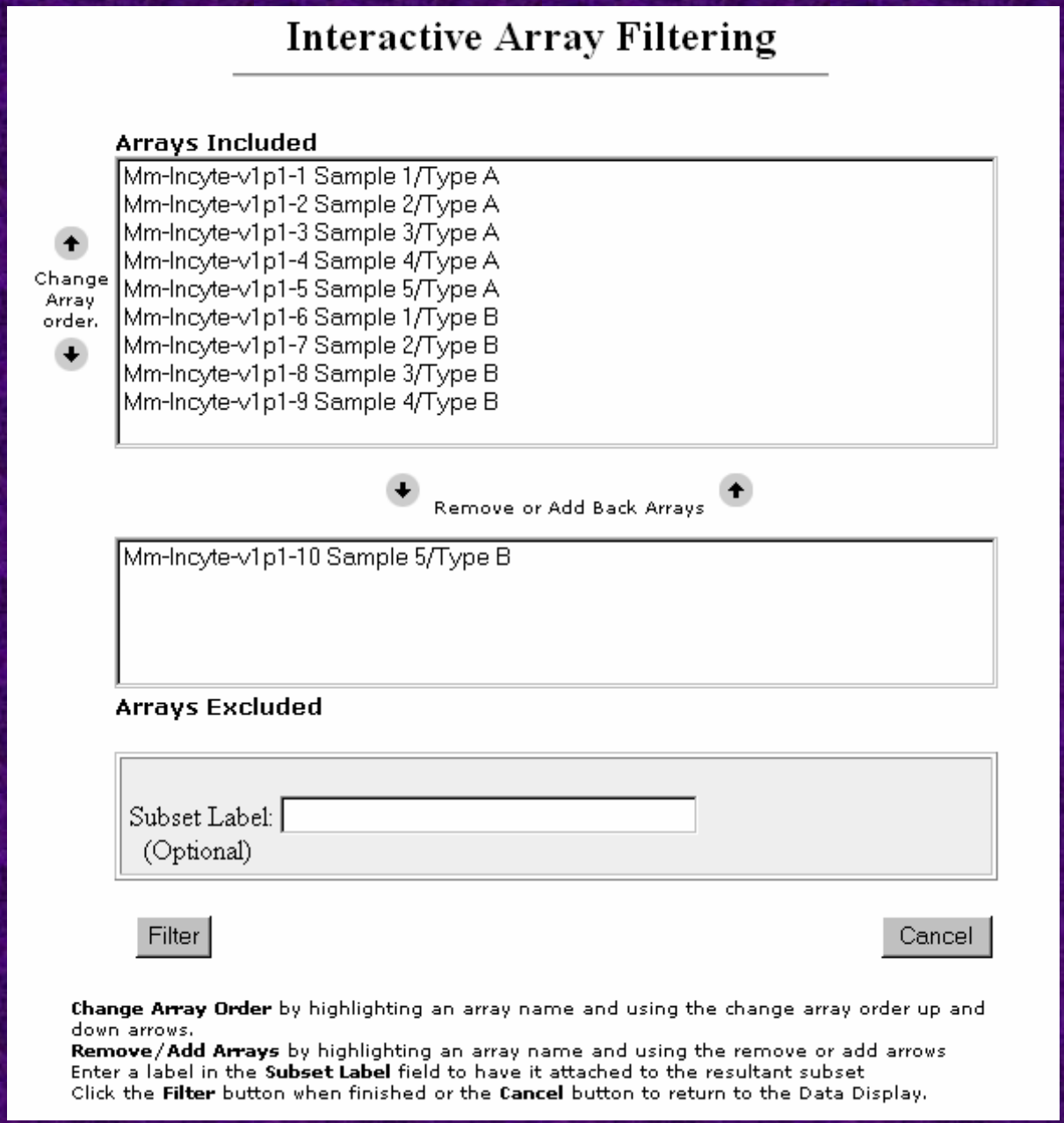


At any time, can save a subset as a new dataset
In effect, this starts the tree of subsets over again at the top…



Dataset Management:

1. Can delete a dataset – but must delete parent and all children!
2. Can promote datasets (Transient to Temporary or Permanent; Temporary to Permanent)

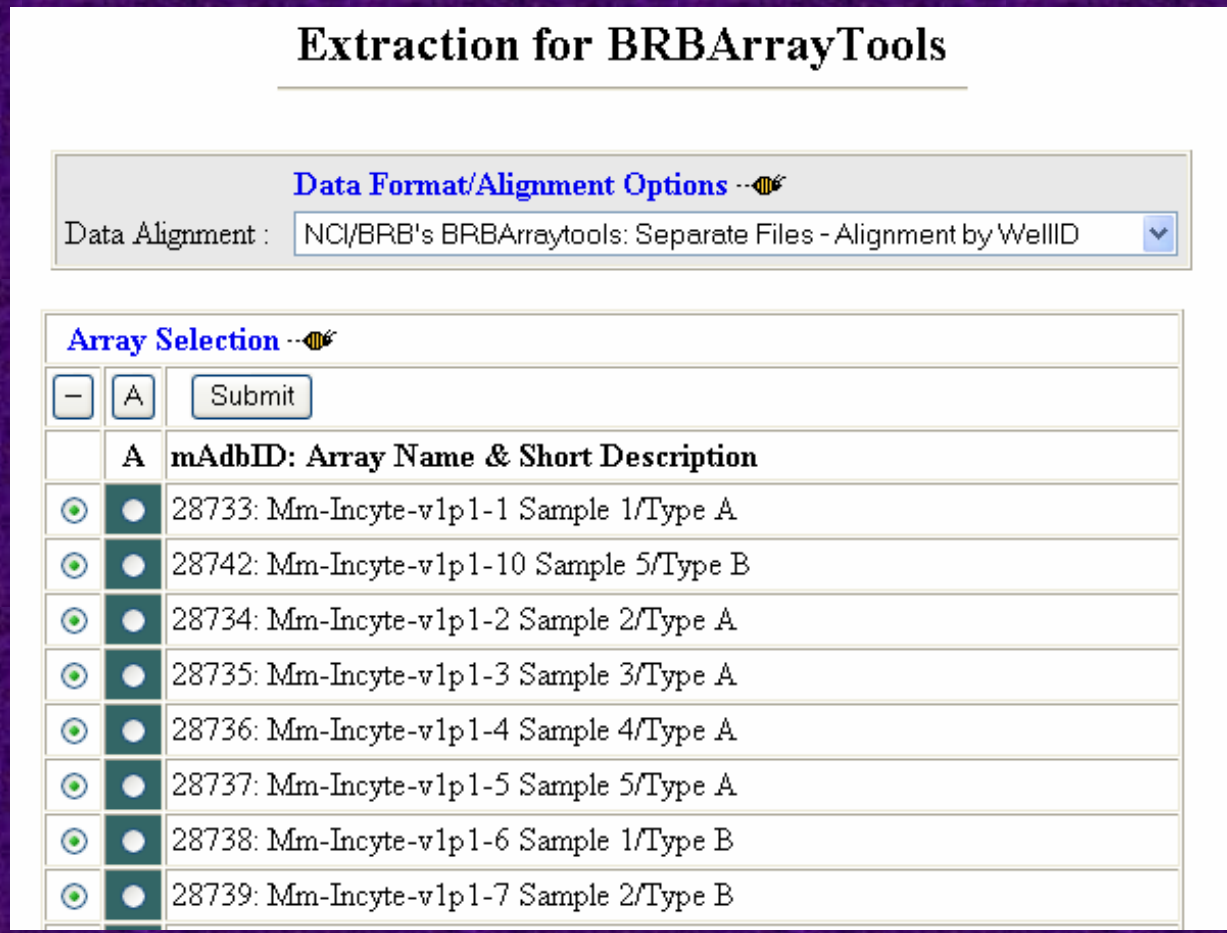


Allows re-ordering and removal of arrays from a subset

Accessed from main pull down list on Data Display Page

Exporting Data to Other Microarray Analysis Tools

- BRB Array tools export by well ID or by UniGene ID
- GeneSpring export
- MA Explorer export



Retrieving Uploaded Data

mAdb: Data Retrieval Form

This tool allows you to retrieve the orignal uploaded data files.

Upload Retrieval Options

Package Format: ZIP file (.zip)

Include: ☑ Image Files (Spotted Uploads only)

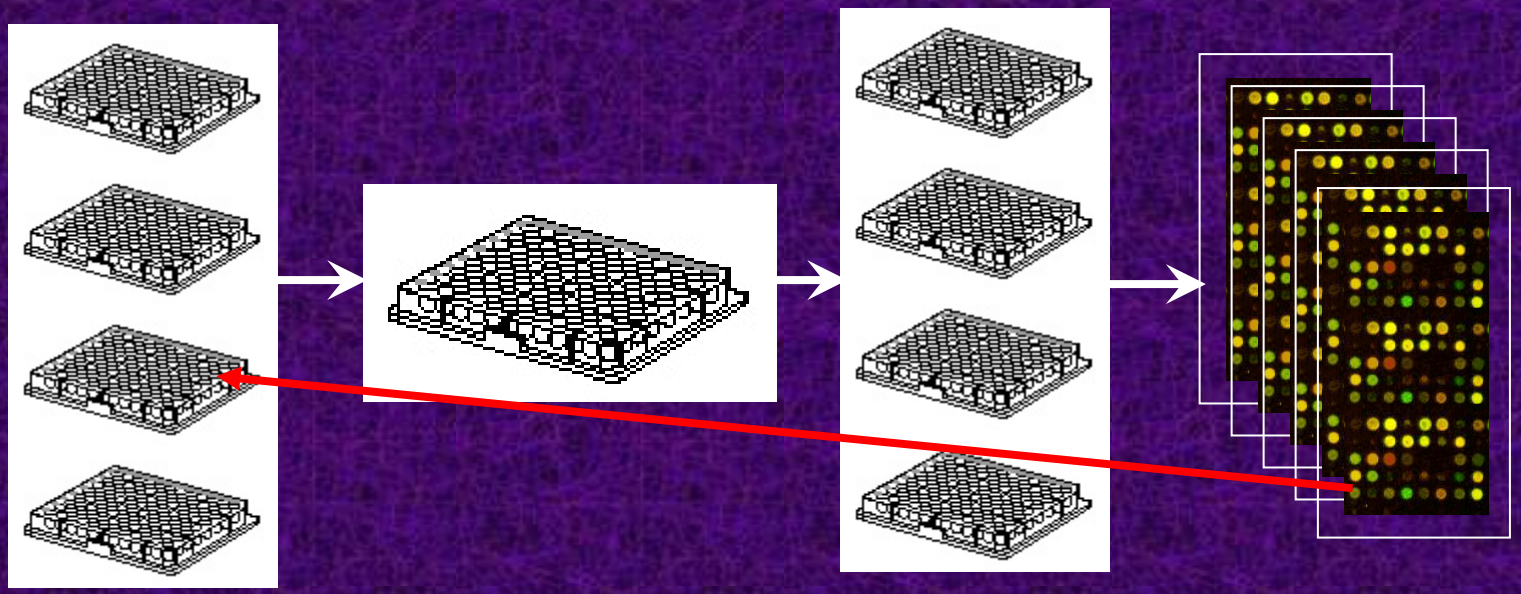
☑ Array Description Files

Array Selection

− | A | Submit

	A	ID #	Array Name & Description
◉	●	28733	Mm-Incyte-v1p1-1 Sample 1/Type A
◉	●	28734	Mm-Incyte-v1p1-2 Sample 2/Type A
◉	●	28735	Mm-Incyte-v1p1-3 Sample 3/Type A
◉	●	28736	Mm-Incyte-v1p1-4 Sample 4/Type A
◉	●	28737	Mm-Incyte-v1p1-5 Sample 5/Type A

mAdb Database Design: Feature Tracking



- mAdb works with microarray facilities to track printing from arrays back to inventory plates
- Allows mAdb support staff to correct printing errors

mAdb Tips for array analysis

- Always look at Project Summaries – normalization factor for a "good" array should be between 0.5 and 2.0.
- If you have replicate arrays (and you should), do a scatter plot to determine the correlation between the arrays (i.e. how close the slope is to 1. For reverse fluors, how close to –1) just for QC purposes.
- Turning **Show Spot Images** off, generally displays results faster – only need for spot QA.

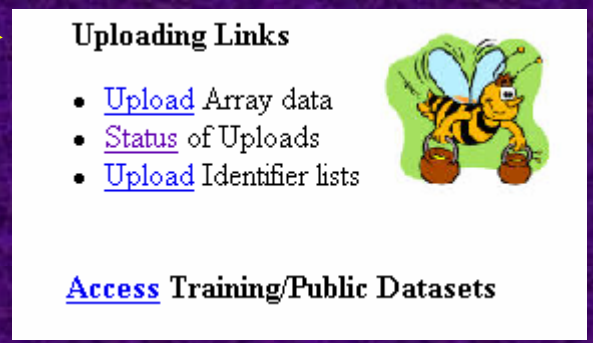
General tips for array analysis

At a recent Microarray Data Analysis conference in Washington D.C., several speakers laid out what distinguishes a good microarray experiment from a bad one:

- When possible, consult a statistician before you even design your experiment - they offer more than just analysis tools.
- Do a power analysis to determine the number of replicates (i.e. chips) you need to detect an effect. To estimate the effect size, you might want to run a pilot study first or obtain the estimate from previous similar experiments. Regardless of the power analysis results, obtain at least three replicates on different slides or chips.
- Find sources of technical variation before you embark on a hunt for biological effects and standardize your protocols.
- Randomize your variables: for example, don't run all your treatment slides on one day and all your controls on the next.
- Microarray analysis is still a screening tool – confirm your observation by other methods – RT-PCR, Northern blot, protein levels
- See **http://linus.nci.nih.gov/~brb/TechReport.htm** for good references on design, analysis issues, and myths/truths

Other microarray training:

- Hands-on analysis tool mAdb class #412 – next available class on November 16-17, 1 - 4 PM
- Statistical Analysis of Microarray Data & BRB Array Tools (from the NCI Biometrics Research Branch) class #410 offered bimonthly; next class October 26-27, 12:30-4:30PM
- Partek Pro, R, GeneSpring classes – training.cit.nih.gov
- Sample datasets to try out the system are available from a link on the Gateway Page



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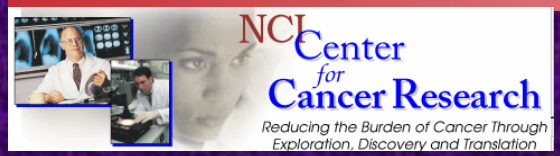
- Jim Tomlin
- Esther Asaki*
- John Greene, Ph.D.*
- Kathleen Meyer*
- Tim Ruppert*

*SRA International contractor



http://madb.nci.nih.gov
http://madb.niaid.nih.gov

For assistance, remember:

madb_support@bimas.cit.nih.gov

Thank you!!